

0965264-09304  
T09260-4929960

```
Inversion start site
|
ATTATAAAGGAAAAAGAAAATAACGCAATGGACAAGTGGTG
860 +-----+-----+-----+-----+ 900
    TAATATTTCTTTTTCTTTATTGCGTTACCTGTTCAACCAC (41)
      Y K G K R K * R N G Q V V

AAGCTGTGAACTCAGGTGTGCACAATTATCAGGAACACCCCAAACCAAAGTGAGGTAGA
901 -----+-----+-----+-----+-----+ 960
    TTCGACACTTGAGTCCACACGTGTTAATAGTCCTTGTGGGGTTTGGTTTCACTCCATCT (101)
      K L * T Q V C T I I R N T P K P K * G R

AATAGCATGAGAAGCCGTGTTTGATGTTAATTAATT
961 -----+-----+-----+----- 996
    TTATCGTACTCTTCGGCACAACTACAATTAATTAA (137)
      N S M R S R V * C * L I
```

**The inversion sequence of the apo-dystrophin-4 cDNA (SEQ ID NO 1)**

**Figure 1**

Inversion start site  
|

```

850 TAAAGAAAGAATTATAAAGGAAAAAGAAAATAACGCAATGGACAAGTGGTG
    +-----+-----+-----+-----+-----+
    ATTTCTTTCTTAATATTTCTTTTCTTTTATTGCGTTACCTGTTCAACCAC (51)
    * R K N Y K G K R K * R N G Q V V

    AAGCTGTGAACTCAGGTGTGCACAATTATCAGGAACACCCCAAACCAAAGTGAGGTAGA
901 -----+-----+-----+-----+-----+ 960
    TTCGACACTTGAGTCCACACGTGTTAATAGTCCTTGTGGGGTTTGGTTTCACTCCATCT (111)
    K L * T Q V C T I I R N T P K P K * G R

    AATAGCATGAGAAGCCGTGTTTGATGTTAATTAATT
961 -----+-----+-----+----- 996
    TTATCGTACTCTTCGGCACAACTACAATTAATTAA (147)
    N S M R S R V * C * L I
  
```

**The inversion sequence of the apo-dystrophin-4 cDNA plus a 10 base-pair region 5' to the start of the inversion sequence (SEQ ID NO 1A).**

**Figure 1A**

0996364-09301

```

Start at 710
|
ACAATGGCAG
+-----+ 720
TTGTTACCGTC (11)
  Q  W  Q

721  GTTTTACACGTCTATGCAATTGTACAAAAAGTTATAAGAAAACCTACATGTAAAATCTTG
-----+-----+-----+-----+-----+-----+-----+ 780
CAAATGTGCAGATACGTTAACATGTTTTTCAATATTCTTTGATGTACATTTTAGAAC (71)
V  L  H  V  Y  A  I  V  Q  K  S  Y  K  K  T  T  C  K  I  L

781  ATAGCTAAATAACTTGCCATTTCTTTATATGGAACGCATTTTGGGTGTTTAAAAATTTA
-----+-----+-----+-----+-----+-----+ 840
TATCGATTTATTGAACGGTAAAGAAATATACCTTGCCTAAAACCCAACAAATTTTAAAT (131)
I  A  K  *  L  A  I  S  L  Y  G  T  H  F  G  L  F  K  N  L
              inversion start site
              |
841  TAACAGTTATAAAGAAAGAATTATAAAGGAAAAAGAAAATAACGCAATGGACAAGTGGTG
-----+-----+-----+-----+-----+-----+ 900
ATTGTCAATATTTCTTTCTTAATATTTCTTTTCTTTTATTGCGTTACCTGTTCAACCAC (191)
*  Q  L  *  R  K  N  Y  K  G  K  R  K  *  R  N  G  Q  V  V

901  AAGCTGTGAACTCAGGTGTGCACAATTATCAGGAACACCCCAAACCAAAGTGAGGTAGA
-----+-----+-----+-----+-----+-----+ 960
TTCGACACTTGAGTCCACACGTGTTAATAGTCCTTGTGGGGTTTGGTTTCACTCCATCT (251)
K  L  *  T  Q  V  C  T  I  I  R  N  T  P  K  P  K  *  G  R

961  AATAGCATGAGAAGCCGTGTTTGATGTTAATTAATT
-----+-----+-----+-----+-----+ 996
TTATCGTACTCTTCGGCACAACTACAATTAATTAA (287)
N  S  M  R  S  R  V  *  C  *  L  I

```

**The inversion sequence of the apo-dystrophin-4 cDNA plus the upstream 150 bp from the start of the inversion at 860 to the Hpa I enzyme site (SEQ ID NO 1B)**

**Figure 1B**



```

GGGAATGATTTCCCAAATGGCAAAGAAACAGAGTGATGCTATCTATCTGCACCTTTTGTA
361 -----+-----+-----+-----+-----+-----+ 420
CCCTTACTAAAGGGTTTACCGTTTCTTTGTCTCACTACGATAGATAGACGTGGAAAACAT
G N D F P N G K E T E * C Y L S A P F V

AAGTCTGTCTTTCTTTCTCTTTGTTTTCCAGGACACAATGTAGGAAGTCTTTTCCACATG
421 -----+-----+-----+-----+-----+-----+ 480
TTCAGACAGAAAGAAAGAGAAACAAAAGGTCTGTGTTACATCCTTCAGAAAAGGTGTAC
K S V F L S L C F P G H N V G S L F H M

GCAGATGATTTGGGCAGAGCGATGGAGTCCTTAGTATCAGTCATGACAGATGAAGAAGGA
481 -----+-----+-----+-----+-----+-----+ 540
CGTCTACTAAACCCGTCTCGCTACCTCAGGAATCATAGTCAGTACTGTCTACTTCTTCCT
A D D L G R A M E S L V S V M T D E E G

GCAGAATAAATGTTTTACAACCTCCTGATTCCCGCATGGTTTTTATAATATTCATACAACA
541 -----+-----+-----+-----+-----+-----+ 600
CGTCTTATTTACAAAATGTTGAGGACTAAGGGCGTACCAAAAATATTATAAGTATGTTGT
A E * M F Y N S * F P H G F Y N I H T T

AAGAGGATTAGACAGTAAGAGTTTACAAGAAATAAATCTATATTTTTGTGAAGGGTAGTG
601 -----+-----+-----+-----+-----+-----+ 660
TTCTCCTAATCTGTCATTCTCAAATGTTCTTTATTTAGATATAAAAACACTTCCCATCAC
K R I R Q * E F T R N K S I F L * R V V

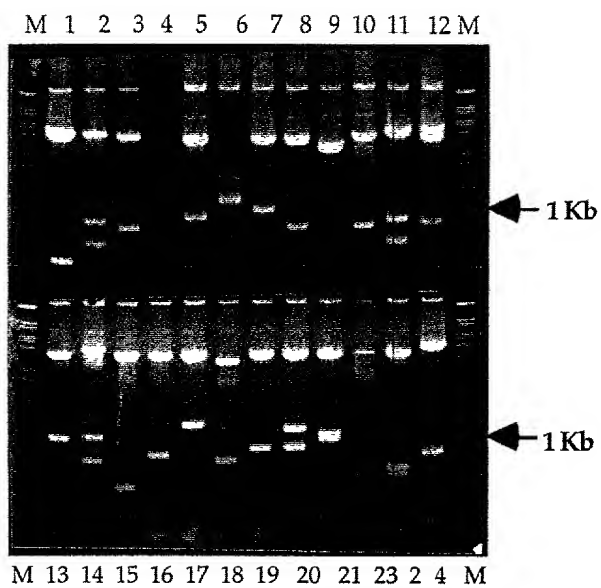
GTATTATACTGTAGATTTCACTAGTTTCTAAGTCTGTTATTGTTTTGTTAACAATGGCAG
661 -----+-----+-----+-----+-----+-----+ 720
CATAATATGACATCTAAAGTCATCAAAGATTTCAGACAATAACAAAACAATTGTTACCGTC
V L Y C R F Q * F L S L L L F C * Q W Q

```

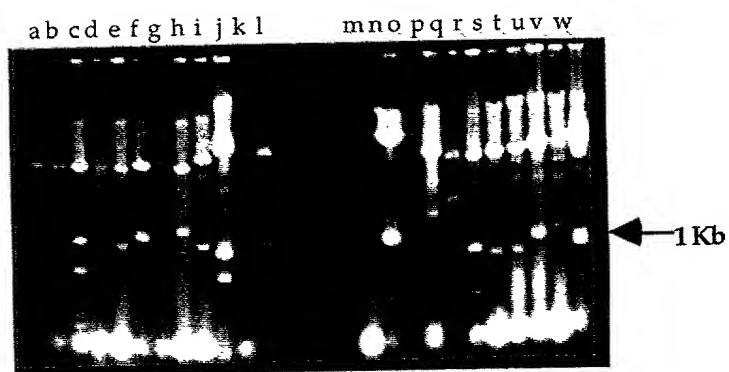
**Figure 2 (cont'd)**

GTTTTACACGTCTATGCAATTGTACAAAAAAGTTATAAGAAAACCTACATGTAAAATCTTG  
 721 -----+-----+-----+-----+-----+-----+ 780  
 CAAAATGTGCAGATACGTTAACATGTTTTTCAATATTCTTTTGATGTACATTTTAGAAC  
 V L H V Y A I V Q K S Y K K T T C K I L  
  
 ATAGCTAAATAACTTGCCATTTCTTTATATGGAACGCATTTTGGGTTGTTTAAAAATTTA  
 781 -----+-----+-----+-----+-----+-----+ 840  
 TATCGATTTATTGAACGGTAAAGAAATATACCTTGCGTAAAACCCAACAAATTTTAAAT  
 I A K \* L A I S L Y G T H F G L F K N L  
  
 TAACAGTTATAAAGAAAGATTATAAAGGAAAAAGAAAATAACGCAATGGACAAGTGGTG  
 841 -----+-----+-----+-----+-----+-----+ 900  
 ATTGTCAATATTTCTTTCTTAATATTTCTTTCTTTTATTGCGTTACCTGTTCCACCAC  
 \* Q L \* R K N Y K G K R K \* R N G Q V V  
  
 AAGCTGTGAACTCAGGTGTGCACAATTATCAGGAACACCCCAAAACCAAAGTGAGGTAGA  
 901 -----+-----+-----+-----+-----+-----+ 960  
 TTCGACACTTGAGTCCACACGTGTTAATAGTCCTTGTTGGGGTTTGGTTTCACTCCATCT  
 K L \* T Q V C T I I R N T P K P K \* G R  
  
 AATAGCATGAGAAGCCGTGTTTGATGTTAATTAATT  
 961 -----+-----+-----+----- 996  
 TTATCGTACTCTTCGGCACAACTACAATTAATTAA  
 N S M R S R V \* C \* L I

Figure 2 (cont'd)



**Figure 3A**



**Figure 3B**

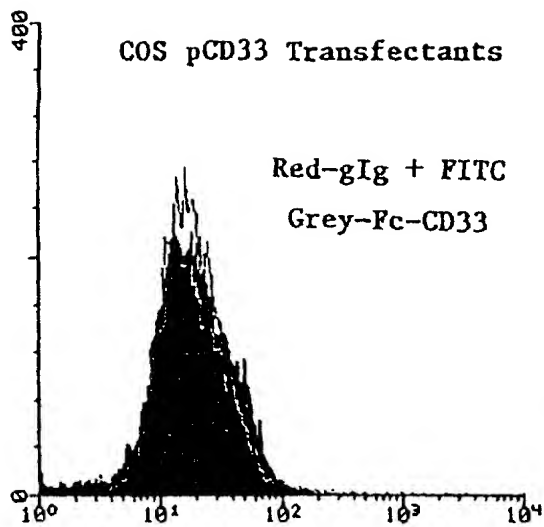


Figure 4A

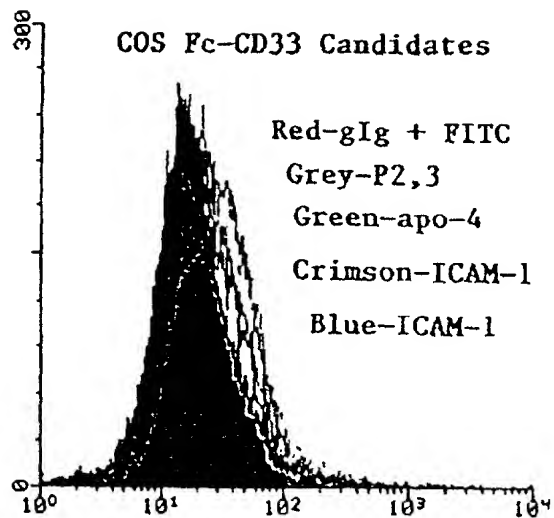


Figure 4B

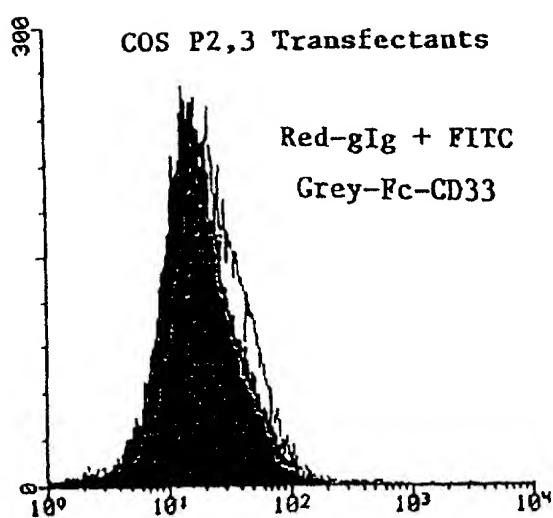


Figure 4C

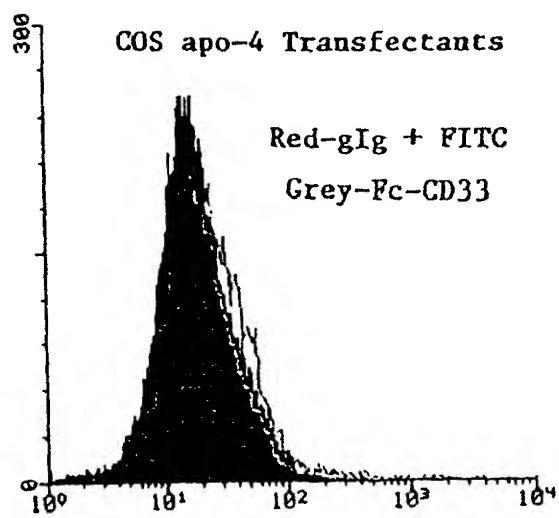


Figure 4D



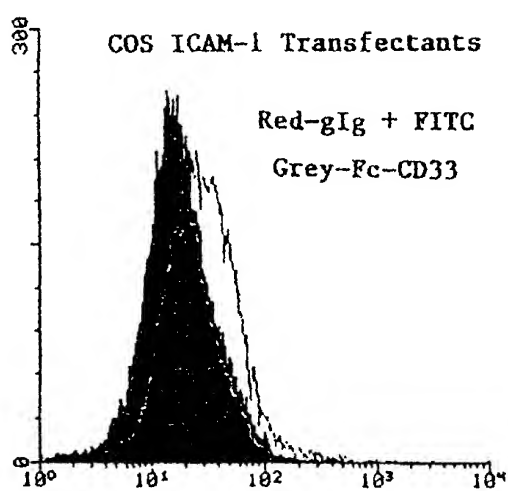


Figure 4E

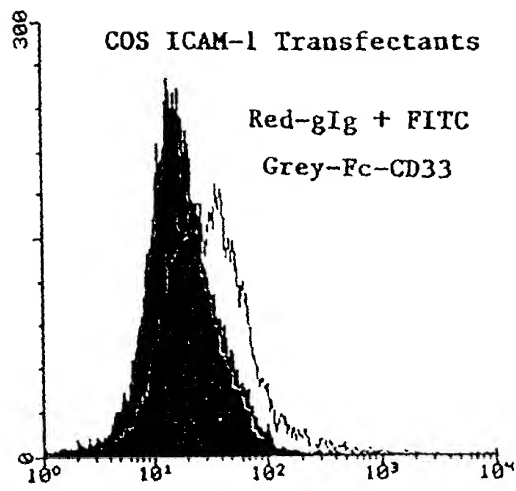


Figure 4F

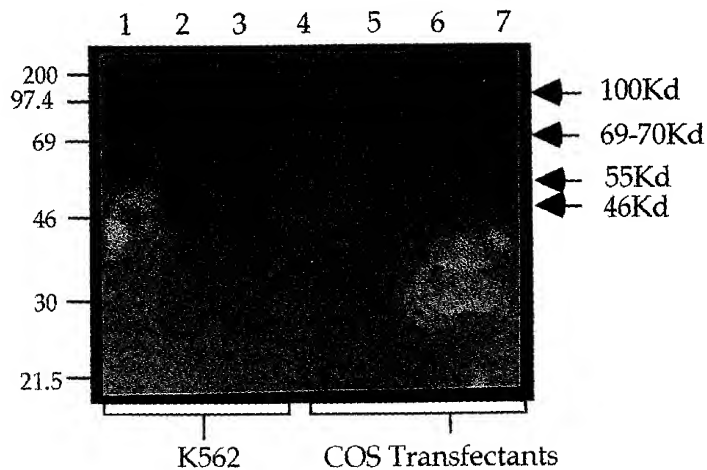


Figure 5



begin exon 79  
|

421 AAGTCTGTCTTTCTTTCTCTTTGTTTTCCAGGACACAATGTAGGAAGTCTTTTCCACATG 480  
-----+-----+-----+-----+-----+-----+  
TTCAGACAGAAAGAAAGAGAAACAAAAGGTCCTGTGTACATCCTTCAGAAAAGGTGTAC  
K S V F L S L C F P G H N V G S L F H M

481 GCAGATGATTTGGGCAGAGCGATGGAGTCCTTAGTATCAGTCATGACAGATGAAGAAGGA 540  
-----+-----+-----+-----+-----+-----+  
CGTCTACTAAACCCGTCTCGCTACCTCAGGAATCATAGTCAGTACTGTCTACTTCTTCCT  
A D D L G R A M E S L V S V M T D E E G

541 GCAGAATAAATGTTTTACAACCTCCTGATTCCCGCATGGTTTTTATAATATTCATACAACA 600  
-----+-----+-----+-----+-----+-----+  
CGTCTTATTTACAAAATGTTGAGGACTAAGGGCGTACCAAAAATATTATAAGTATGTTGT  
A E \* M F Y N S \* F P H G F Y N I H T T

(----N----)

601 AAGAGGATTAGACAGTAAGAGTTTACAAGAAATAAATCTATATTTTGTGAAGGGTAGTG 660  
-----+-----+-----+-----+-----+-----+  
TTCTCCTAATCTGTCAATTCTCAAATGTTCTTTATTTAGATATAAAAACACTTCCCATCAC  
K R I R Q \* E F T R N K S I F L \* R V V

661 GTATTATACTGTAGATTTCAAGTCTGTTATTGTTTTGTTAACAATGGCAG 720  
-----+-----+-----+-----+-----+-----+  
CATAATATGACATCTAAAGTCATCAAAGATTGAGACAATAACAAAACAATTGTTACCGTC  
V L Y C R F Q \* F L S L L L F C \* Q W Q

721 GTTTTACACGTCTATGCAATTGTACAAAAAGTTATAAGAAAACCTACATGTAAAACTCTTG 780  
-----+-----+-----+-----+-----+-----+  
CAAAATGTGCAGATACGTTAACATGTTTTTTCAATATTCTTTTGATGTACATTTTAGAAC  
V L H V Y A I V Q K S Y K K T T C K I L

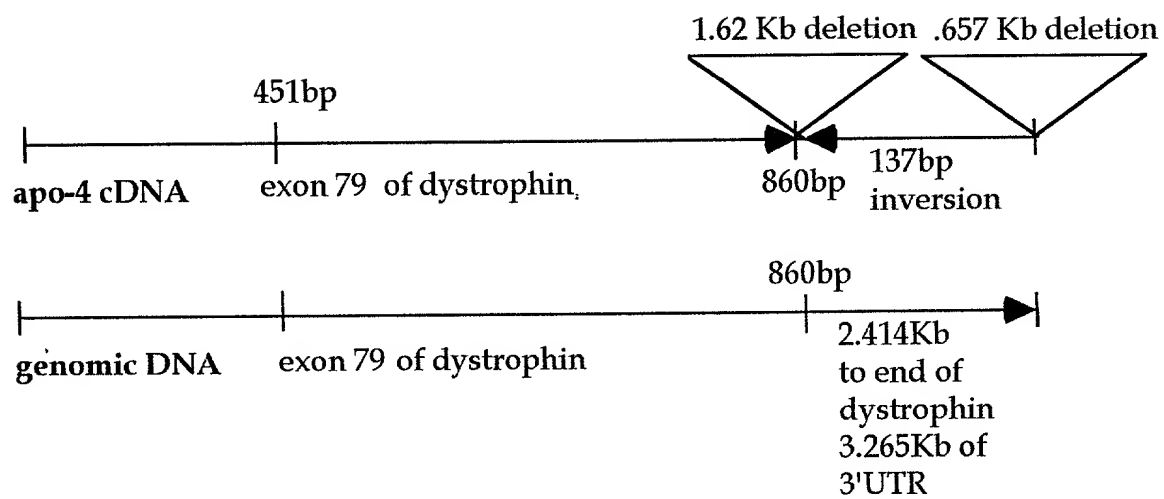
781 ATAGCTAAATAACTTGCCATTTCTTTATATGGAACGCATTTTGGGTGTTTAAAAATTTA 840  
-----+-----+-----+-----+-----+-----+  
TATCGATTTATTGAACGGTAAAGAAATATACCTTGCGTAAACCCAACAAATTTTAAAT  
I A K \* L A I S L Y G T H F G L F K N L  
inversion start site  
|

841 TAACAGTTATAAAGAAAGAATTATAAAGGAAAAAGAAAATAACGCAATGGACAAGTGGTG 900  
-----+-----+-----+-----+-----+-----+  
ATTGTCAATATTTCTTCTTAATATTTCTTTTCTTTTATTGCGTTACCTGTTCCACCAC  
\* Q L \* R K N Y K G K R K \* R N G Q V V

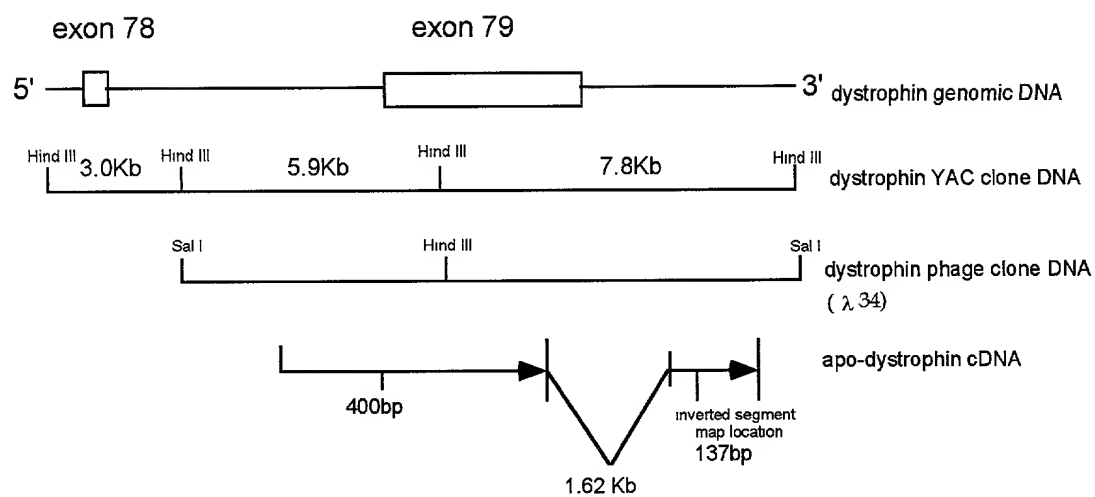
901 AAGCTGTGAACCTCAGGTGTGCACAATTATCAGGAACACCCCAAACCAAGTGAGGTAGA 960  
-----+-----+-----+-----+-----+-----+  
TTCGACACTTGAGTCCACACGTGTTAATAGTCCTTGTGGGGTTTTGGTTTCACTCCATCT  
K L \* T Q V C T I I R N T P K P K \* G R

961 AATAGCATGAGAAGCCGTGTTTGATGTTAATTAATT 996  
-----+-----+-----+-----+  
TTATCGTACTCTTCGGCACAACTACAATTAATTAA  
N S M R S R V \* C \* L I

Figure 6 (cont'd)



**Figure 7**



\*cDNA map is not precisely drawn to scale

**Figure 8**

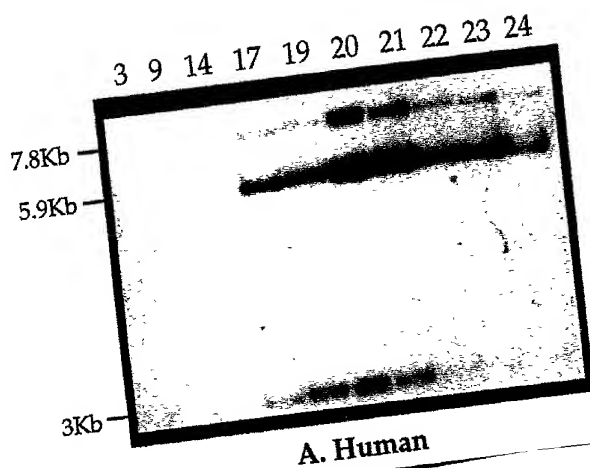


Figure 9A

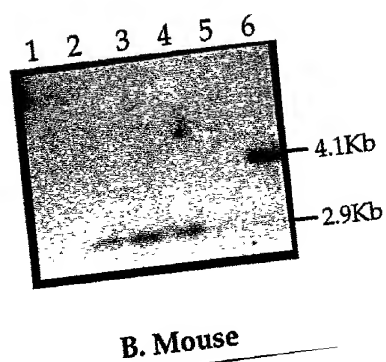


Figure 9B

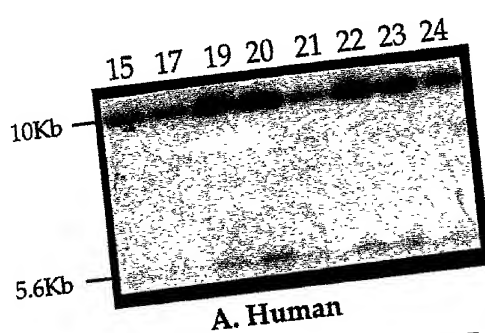


Figure 10A

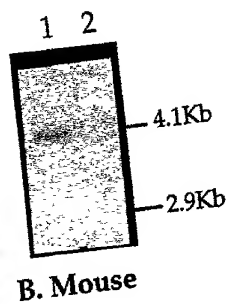


Figure 10B

0906264 092601

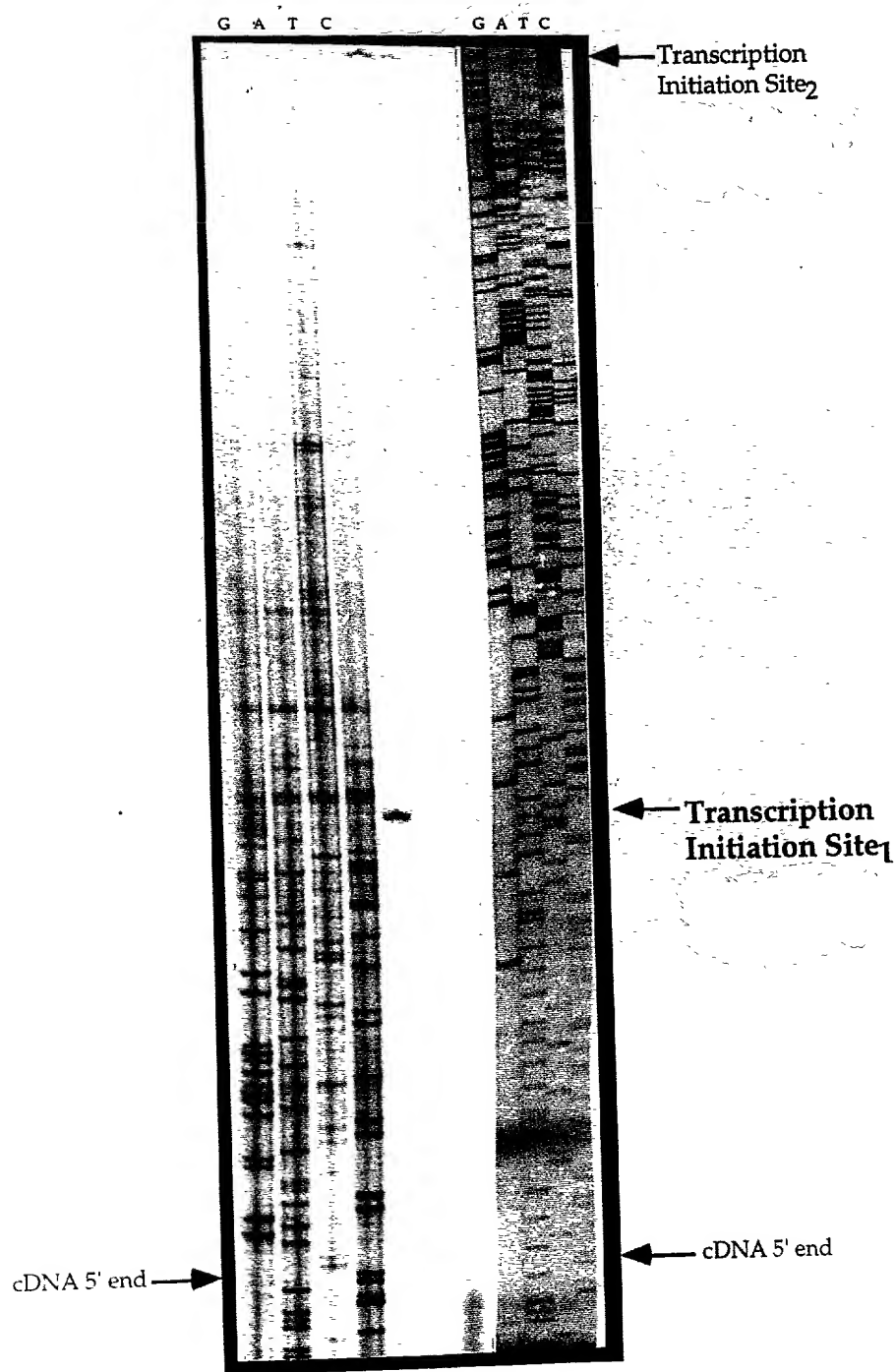
	50				1
Mgen1073	.....	.....	.....	.....	.....
Hapo1234	ctagtttctt	attcaatgta	tagtgcacca	aaggtcaatt	caagagttta
Consensus	-----	-----	-----	-----	-----
	51				100
Mgen1073	.....	.....	.....	.....	.....
Hapo1234	ttattattat	tttcaaccga	agtaaaagca	gagagaaaat	agccacctcc
Consensus	-----	-----	-----	-----	-----
	101			begin GRAIL exon @149 150	
Mgen1073	.....	.....	ttcACAGgCT	tAAgCAGCca	gtAAATGAcA
Hapo1234	accatagcct	cagaagcaag	ccaACAGcCT	gAAaCAGCtt	tgAAATGAaA
Consensus	-----	-----	---ACAG-CT	-AA-CAGC--	--AAATGA-A
	151				200
Mgen1073	AtT.....	.....	.....T	AtgtGgtAgt	cAgGtcactG
Hapo1234	AgTtgggtgtg	gcgggtgatgg	tggcagtgaT	AatgGtgAcc	gAtGgttggG
Consensus	A-T-----	-----	-----T	A---G--A--	-A-G-----G
	201			apo-4 5'end 250	
Mgen1073	TGCTGGTaat	GGTgaTctTA	GcaGgcAgAG	aaGGTGgTaG	TGaTTTGATa
Hapo1234	TGCTGGTgAT	GGTagTggTA	GttGtgA.AG	gtGGTgaTgG	TGgTTTGATt
Consensus	TGCTGGT-AT	GGT--T--TA	G--G--A-AG	--GGTG-T-G	TG-TTTGAT-
	251	M1			300
Mgen1073	GtaAaagtgt	AgAcTaTaCa	acAgaAtAAa	TAcAagtata	GTAA.....
Hapo1234	GatAgtaaaa	AaAaTgTtCg	ttAatAcAAg	TAgAgagtaa	GTAAatcaatc
Consensus	G--A-----	A-A-T-T-C-	--A--A-AA-	TA-A-----A	GTAA-----
	301		M2	M3	350
Mgen1073	.....atc	caaCAAaGTG	tgAAAGgTGT	gTgCCATtAc	acAtctTTCT
Hapo1234	aatcactcat	agcCAAaGTG	gaAAAGaTGT	aTcCCATcAt	ggAataTTCC
Consensus	-----	---CAA-GTG	--AAAG-TGT	-T-CCAT-A-	--A---TTC-
	351				400
Mgen1073	cG.....	GtgATAagag	cCTTgTCTAT	GaAgTTC...	TGAgATgTgT
Hapo1234	tGttctgata	GaaATcttgt	gCTTaTCTAT	GgAaTTCttt	TGAtATaTaT
Consensus	-G-----	G--AT-----	-CTT-TCTAT	G-A-TTC---	TGA-AT-T-T
	401				450
Mgen1073	TaggAagatG	AAtCatcAat	TtaCaT....	TTcTcCCcat	cAAAtgaCac
Hapo1234	TtacAttggg	AAcCtgaAtg	TagCtTgaca	TTtTtCCatg	tAAAcacCAg
Consensus	T---A----G	AA-C---A--	T--C-T----	TT-T-CC---	-AAA---CA-
	451		begin mouse GRAIL exon		500
Mgen1073	cAtgCTGATC	CAGtATTAAg	CTaATACTAA	C.....ACca	tgcAatGCTT
Hapo1234	tAgcCTGATC	CAacATTAAg	CTgATACTAA	CaaacaACgt	gtaAtgGCTT
Consensus	-A--CTGATC	CA--ATTAAg	CT-ATACTAA	C-----AC--	---A--GCTT
	501				550
Mgen1073	CATTAAcAAG	GaTTTGCTTC	TTgCTaGAAA	tgGGT..AAA	AaCggACTgT
Hapo1234	CATTAAatAAG	GcTTTGCTTC	TTcCTgGAAA	ctGGTgaAAA	AtCaaACctT
Consensus	CATTAA-AAG	G-TTTGCTTC	TT-CT-GAAA	--GGT--AAA	A-C--AC--T
	551				600
Mgen1073	GgTcTGTAtA	CcTtCaATGC	AGCTTaTGTG	TTGTCTTttc	C..tgAAatG
Hapo1234	GtTgTGTAcA	CCcTCgATGC	AGCTTcTGTG	TTGTCTTcaC	CcagaAAatgG
Consensus	G-T-TGTA-A	CC-TC-ATGC	AGCTT-TGTG	TTGTCTT--C	C----AA--G

**Figure 11**

	601		650
Mgen1073	GtAATGAcTc CCaAtAgtGg cAAccAgggG tacaATaCT. ....TGCA		
Hapo1234	GgAATGATtT CCcAaAtgGc aAagaAacaG agtgATgCTa tctatcTGCA		
Consensus	G-AATGA-T- CC-A-A--G- -AA--A---G ----AT-CT- -----TGCA		
	651		exon79 700
Mgen1073	CacTTTGTAA A....CTCTT TCTTTCTCTT TGTTTTCCAG GACACAATGT		
Hapo1234	CctTTTGTAA AgtctgTCTT TCTTTCTCTT TGTTTTCCAG GACACAATGT		
Consensus	C--TTTGTAA A-----TCTT TCTTTCTCTT TGTTTTCCAG GACACAATGT		
	701		750
Mgen1073	AGGAAGcCTT TTCCACATGG CAGATGATTT GGGCAGAGCG ATGGAGTCCT		
Hapo1234	AGGAAGtCTT TTCCACATGG CAGATGATTT GGGCAGAGCG ATGGAGTCCT		
Consensus	AGGAAG-CTT TTCCACATGG CAGATGATTT GGGCAGAGCG ATGGAGTCCT		
	751		800
Mgen1073	TAGTtTCAGT CATGACAGAT GAAGAAGGAG CAGAATAAAT GTTTTACAAC		
Hapo1234	TAGTaTCAGT CATGACAGAT GAAGAAGGAG CAGAATAAAT GTTTTACAAC		
Consensus	TAGT-TCAGT CATGACAGAT GAAGAAGGAG CAGAATAAAT GTTTTACAAC		
	801		850
Mgen1073	TCCTGATTCC CGCATGGTTT TTATAATATT CgTACAACAA AGAGGATTAG		
Hapo1234	TCCTGATTCC CGCATGGTTT TTATAATATT CaTACAACAA AGAGGATTAG		
Consensus	TCCTGATTCC CGCATGGTTT TTATAATATT C-TACAACAA AGAGGATTAG		
	851		900
Mgen1073	ACAGTAAGAG TTTACAAGAA ATaAAATCTA TATTTTTGTG AAGGGTAGTG		
Hapo1234	ACAGTAAGAG TTTACAAGAA AT.AAATCTA TATTTTTGTG AAGGGTAGTG		
Consensus	ACAGTAAGAG TTTACAAGAA AT-AAATCTA TATTTTTGTG AAGGGTAGTG		
	901		950
Mgen1073	GTAcTATACT GTAGATTTCA GTAGTTTCTA AGTCTGTTAT TGTTTTGTTA		
Hapo1234	GTAtTATACT GTAGATTTCA GTAGTTTCTA AGTCTGTTAT TGTTTTGTTA		
Consensus	GTA-TATACT GTAGATTTCA GTAGTTTCTA AGTCTGTTAT TGTTTTGTTA		
	951		1000
Mgen1073	ACAATGGCAG GTTTTACACG TCTATGCAAT TGTACAAAAA AGTTAaAAGA		
Hapo1234	ACAATGGCAG GTTTTACACG TCTATGCAAT TGTACAAAAA AGTTAtAAGA		
Consensus	ACAATGGCAG GTTTTACACG TCTATGCAAT TGTACAAAAA AGTTA-AAGA		
	1001		1050
Mgen1073	AA...ACATG TAAAATCTTG ATAGCTAAAT AACTTGCCAT TTCTTTATAT		
Hapo1234	AAactACATG TAAAATCTTG ATAGCTAAAT AACTTGCCAT TTCTTTATAT		
Consensus	AA---ACATG TAAAATCTTG ATAGCTAAAT AACTTGCCAT TTCTTTATAT		
			begin inversion@1100
	1051		1100
Mgen1073	GGAACGCATT TTGGGTGTGT TAAAAATTTA TAACAGTTAT AAAGAAAGAt		
Hapo1234	GGAACGCATT TTGGGTGTGT TAAAAATTTA TAACAGTTAT AAAGAAAGaA		
Consensus	GGAACGCATT TTGGGTGTGT TAAAAATTTA TAACAGTTAT AAAGAAAGA-		
	1101		1150
Mgen1073	TgtaAAActaA Agtgtgcttt AtAAAAaAAG ttgtTtataA AaaccctAa		
Hapo1234	TtatAAaggA A.....aa AgAAAAaAAC gcaaTggacA AgtgggtgaAg		
Consensus	T---AA---A A-----A A-AAAA-AA- ----T----A A-----A-		
	1151		1200
Mgen1073	acaaacACaC AcGcacaCAC AcacAcacac AcacaCaCaC AcaCaCaCTG		
Hapo1234	ctgtgaACtC AgGtgtgCAC AattAtcagc AacacCcCaA AacCaAaAgTG		
Consensus	-----AC-C A-G----CAC A---A----- A----C-CA- A--CA-A-TG		
	1201		1243
Mgen1073	AGGcAGcAca ttgtTttGcA ttacTtTagc gTGtatcaTA t..		
Hapo1234	AGGtAGaAat agcaTgaGaA gccgTgTttg aTGttaatTA att		
Consensus	AGG-AG-A-- ----T--G-A ----T-T--- -TGT----TA ---		

Figure 11 (cont'd)





**Figure 12A**

-70 bp from 5' end of apo-4

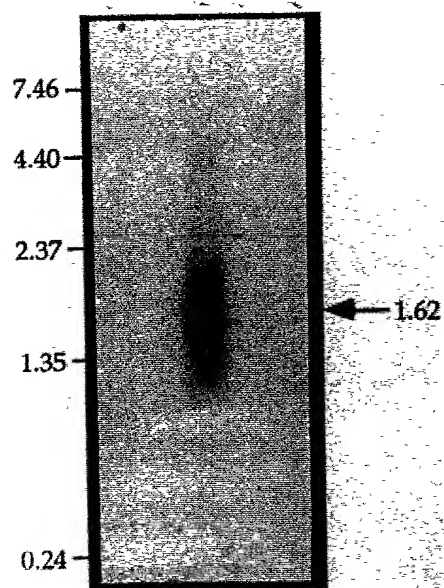
Inr = GCCC TCAT TCTG GAGAC

apo-4 = GCGG TGAT GGTG GCAGT - 48% perfect homology with Inr

71% match on type of base

(purine vs. pyrimidine)

**Figure 12B**



**Figure 13**

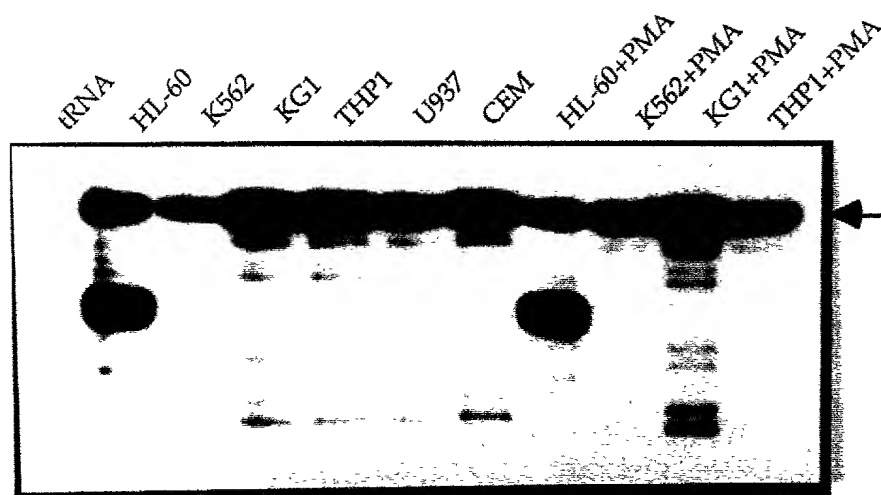
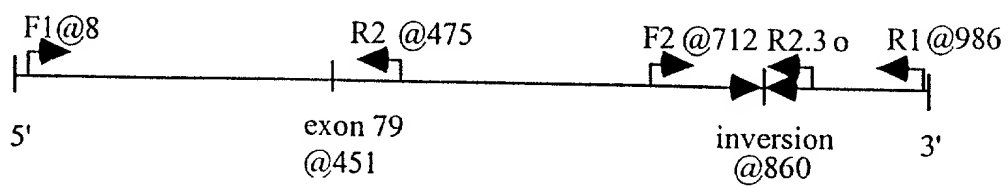


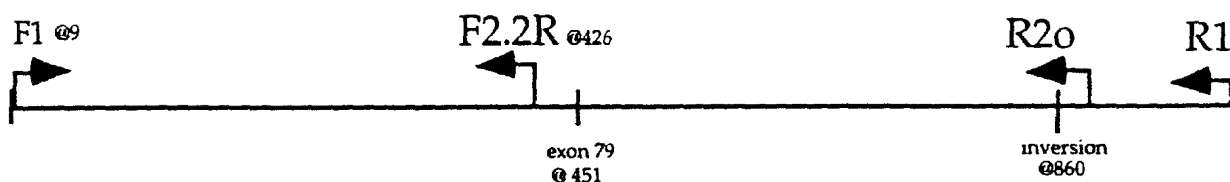
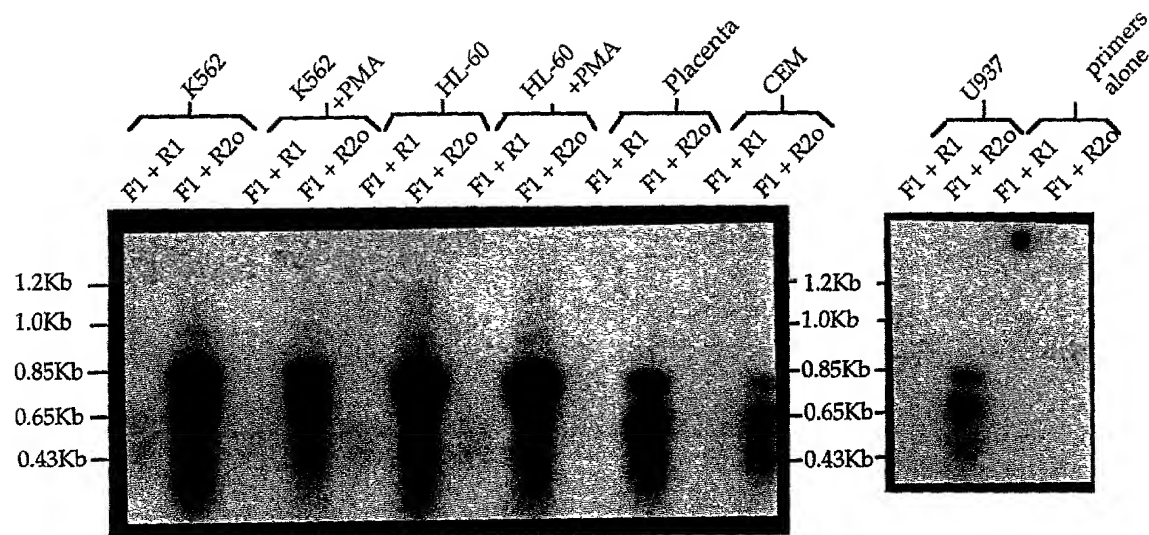
Figure 14



**Figure 15**

0906264-0900  
T03262-49299660

103252 47929553



The Apo-dystrophin cDNA

Figure 16

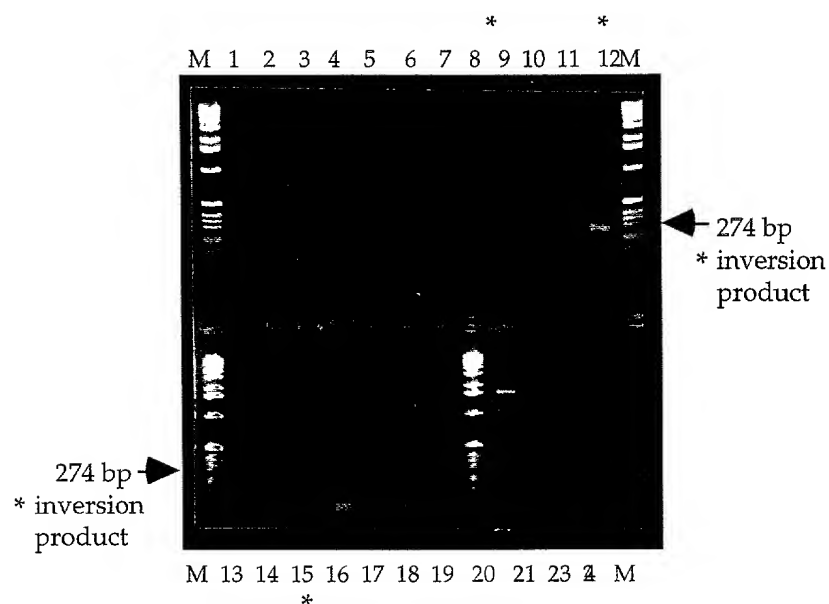


Figure 17A

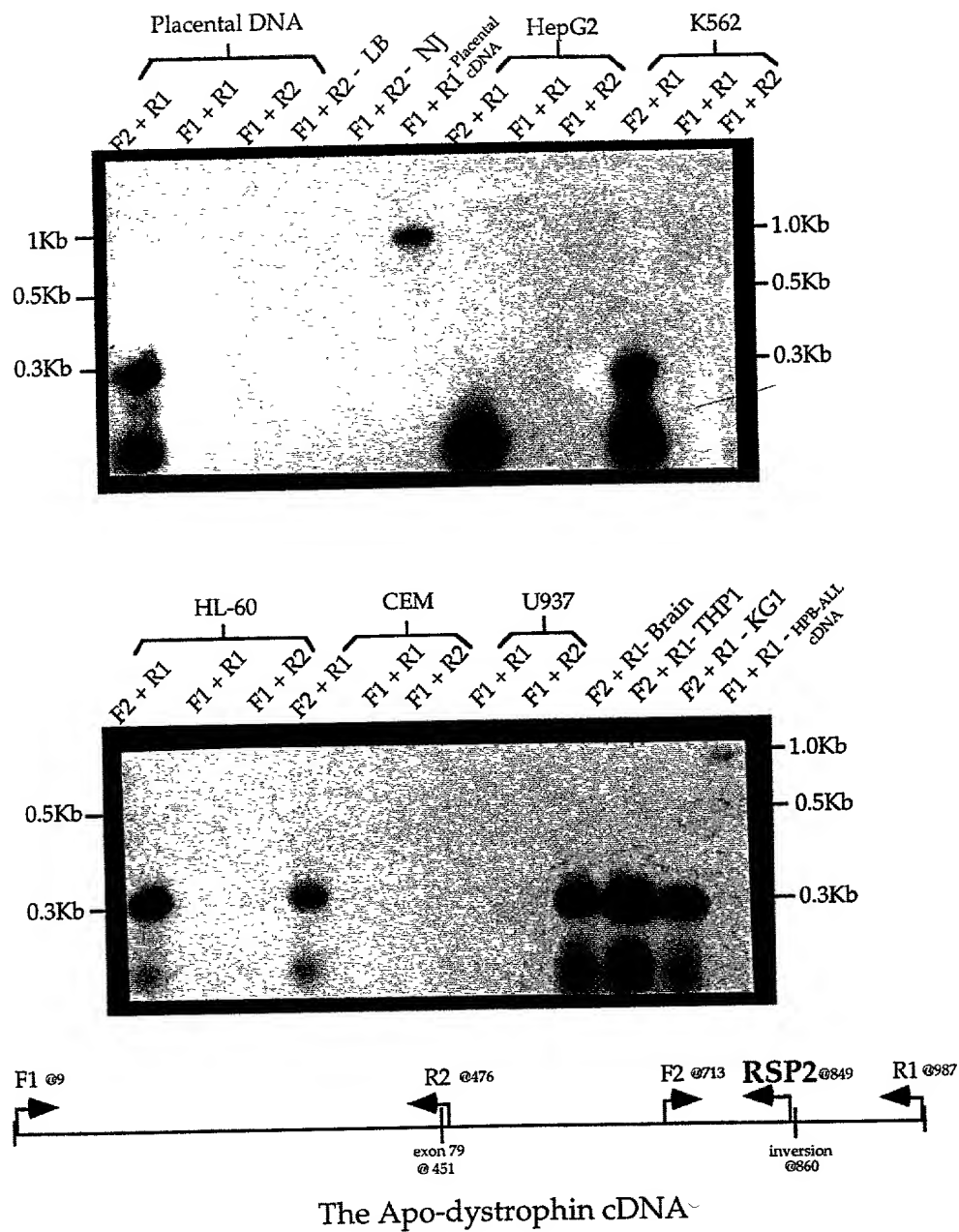


Figure 17B

FOB260-43239660

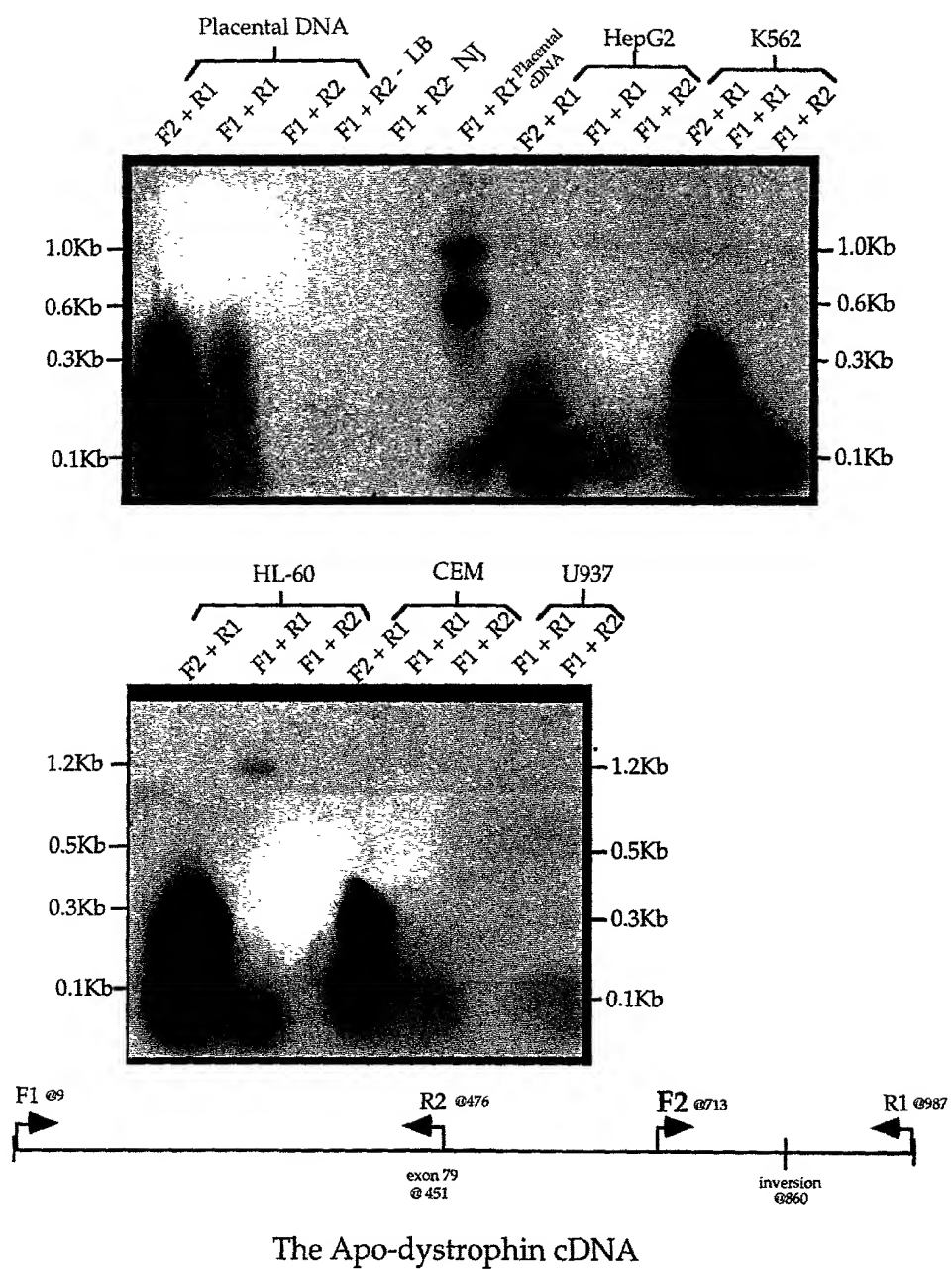


Figure 17C



A.

12/23bp spacer  
CACAGTG-----ACAAAAACC  
heptamer nonamer

Figure 18A

B.

inversion breakpoint<sub>1</sub>

	11640	11650	11660		11670	11680
	*	*	*		*	*
dystrophin	T TTATAACAGT	TATAAAGAAA	GA^TTGTAAAC		<b>TAAAGTGTGC</b>	
	A AATATTGTCA	ATATTTCTTT	CT^AACATTTG		ATTTCACACG	
			a			
apo-4 cDNA	840	850			870	
[ 138 ]	T TTATAACAGT	TATAAAGAAA	GA^TTaTAAAg		gAAAaaGaaa>	
	^ ^^^^^^^^^	^ ^^^^^^^^^	^^ ^v^v^v^v		v^v^v^v^v^v	
dystrophin	T TTATAACAGT	TATAAAGAAA	GA^TTGTAAAC		TAAAGTGTGC	

---

	11690	11700	11710	11720	11730
	*	*	*	*	*
dystrophin	TTTATAAAAA	AAAGTTGTTT	<b>ATAAAAACCC</b>	<b>CTAAAAACAA</b>	AACAAACACA
	AAATATTTTT	TTTCAACAAA	TATTTTTTGGG	GATTTTTTGT	TTGTTTGTGT
apo-4 cDNA	880	890	900	910	920
[ 138 ]	aTaAaAtggA	cAAGTgGTga	ATgtgAACTc	aggtgtgCAc	AAttAtCAGg>
	v^v^v^v^v^v	v^v^v^v^v^v	^^v^v^v^v^v	v^v^v^v^v^v	^^v^v^v^v^v
dystrophin	TTTATAAAAA	AAAGTTGTTT	ATAAAAACCC	CTAAAAACAA	AACAAACACA

---

	11740	11750
	*	*
dystrophin	CACACACACA	<b>CATACACACA</b>
	GTGTGTGTGT	GTATGTGTGT
apo-4 cDNA	940	950
[ 138 ]	aACAC-CcCA	-AaAC-CAaA>
	v^v^v^v^v^v	^v^v^v^v^v
dystrophin	CACACACACA	CATACACACA

Figure 18B

09956234-092301

inversion breakpoint2					
	13130	13140	13150	13160	13170
	*	*	*	*	*
dystrophin	AATTAGCTTT	TGGAGAGTGG	GTTTTGTCCA	TTATTAATAA	TTAATTAATT
	TTAATCGAAA	ACCTCTCACC	CAAAACAGGT	AATAATTATT	AATTAATTAA
apo-4					990
					<AATTAATT
					~~~~~
dystrophin					AATTAATT
-----					
	13180	13190	13200	13210	13220
	*	*	*	*	*
dystrophin	AACATCAAAC	ACGGCTTCTC	ATGCTATTTC	TACCTCACTT	TGGTTTTGGG
	TTGTAGTTTG	TGCCGAAGAG	TACGATAAAG	ATGGAGTGAA	ACCAAAACCC
apo-4	980	970	960	950	940
	<AACATCAAAC	ACGGCTTCTC	ATGCTATTTC	TACCTCACTT	TGGTTTTGGG
	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
dystrophin	AACATCAAAC	ACGGCTTCTC	ATGCTATTTC	TACCTCACTT	TGGTTTTGGG
-----					
	13230	13240	13250	13260	13270
	*	*	*	*	*
dystrophin	GTGTTCTCTGA	TAATTGTGCA	CACCTGAGTT	CACAGCTTCA	CCACTTGTCC
	CACAAGGACT	ATTAACACGT	GTGGACTCAA	GTGTCGAAGT	GGTGAACAGG
apo-4	930	920	910	900	890
	<GTGTTCTCTGA	TAATTGTGCA	CACCTGAGTT	CACAGCTTCA	CCACTTGTCC
	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
dystrophin	GTGTTCTCTGA	TAATTGTGCA	CACCTGAGTT	CACAGCTTCA	CCACTTGTCC
-----					
	13280	13290	13300	13310	13320
	*	*	*	*	*
dystrophin	ATTGCGTTAT	TTTCTTTTTT	CTTTATAATT	CTTTCCTTTT	CCTTCATAAT
	TAACGCAATA	AAAGAAAAAG	GAAATATTAA	GAAAGAAAAA	GGAAGTATTA
	inversion breakpoint3				
	880	870	860	850	840
apo-4	<ATTGCGTTAT	TTTCTTTTTT	CTTTATAATT	CTTTCCTTTT	aacTgtTATA
	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
dystrophin	ATTGCGTTAT	TTTCTTTTTT	CTTTATAATT	CTTTCCTTTT	CCTTCATAAT

Figure 18C

056624-092001

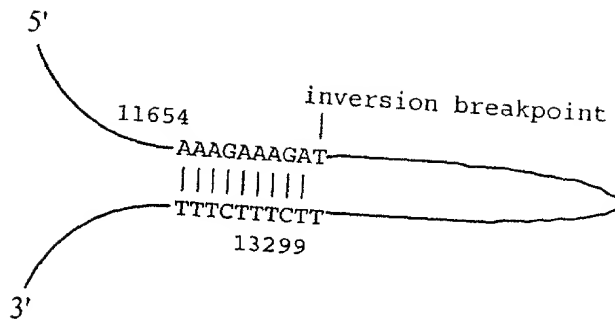
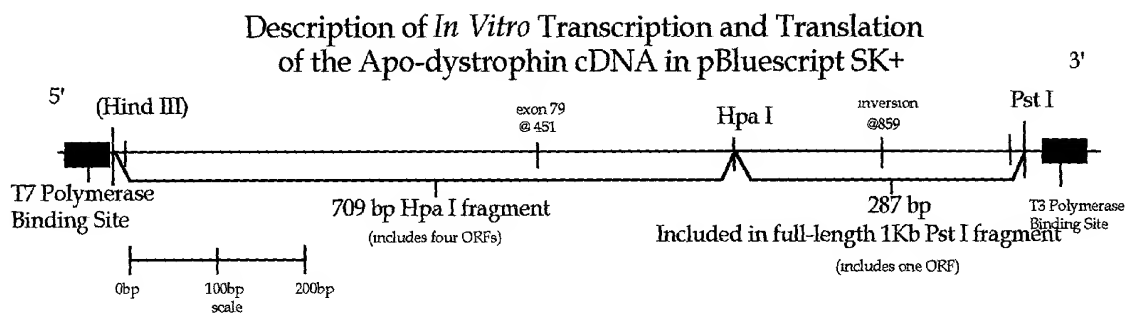


Figure 18D

inversion @ 860

```
TAACAGTATATAAGAAAGAATTATAAGGAAAAAGAAAAATAACGCAATGGACAAGTGGTG
841 -----+-----+-----+-----+-----+-----+-----+ 900
ATTGTCATATTTCTTTCTTAATATTTCTTTTCTTTTATTGCGTTACCTGTTCAACCAC
```

Figure 19

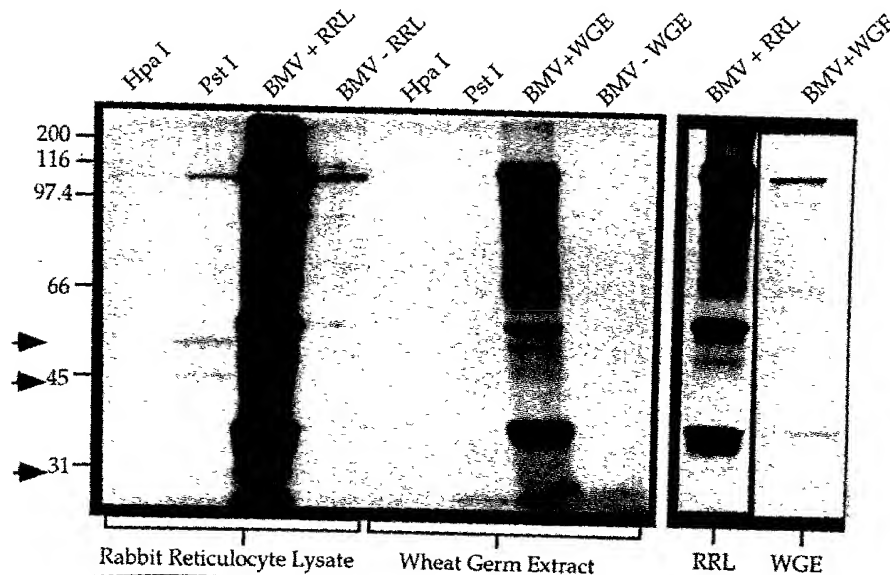


Linearize plasmid with either Hpa I (truncated) or Pst I (full length). Gene Clean and incubate with T7 polymerase and dNTPs to produce RNA *in vitro*.

Incubate RNA with Wheat Germ Extract or Rabbit Reticulocyte Lysates to produce *in vitro* translation

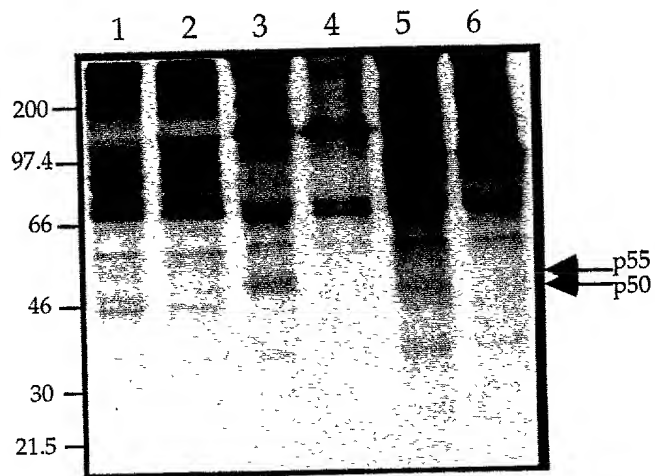
Separate translation products by SDS-PAGE. Fix, Amplify and Dry Gel. Perform Autoradiography

**Figure 20**

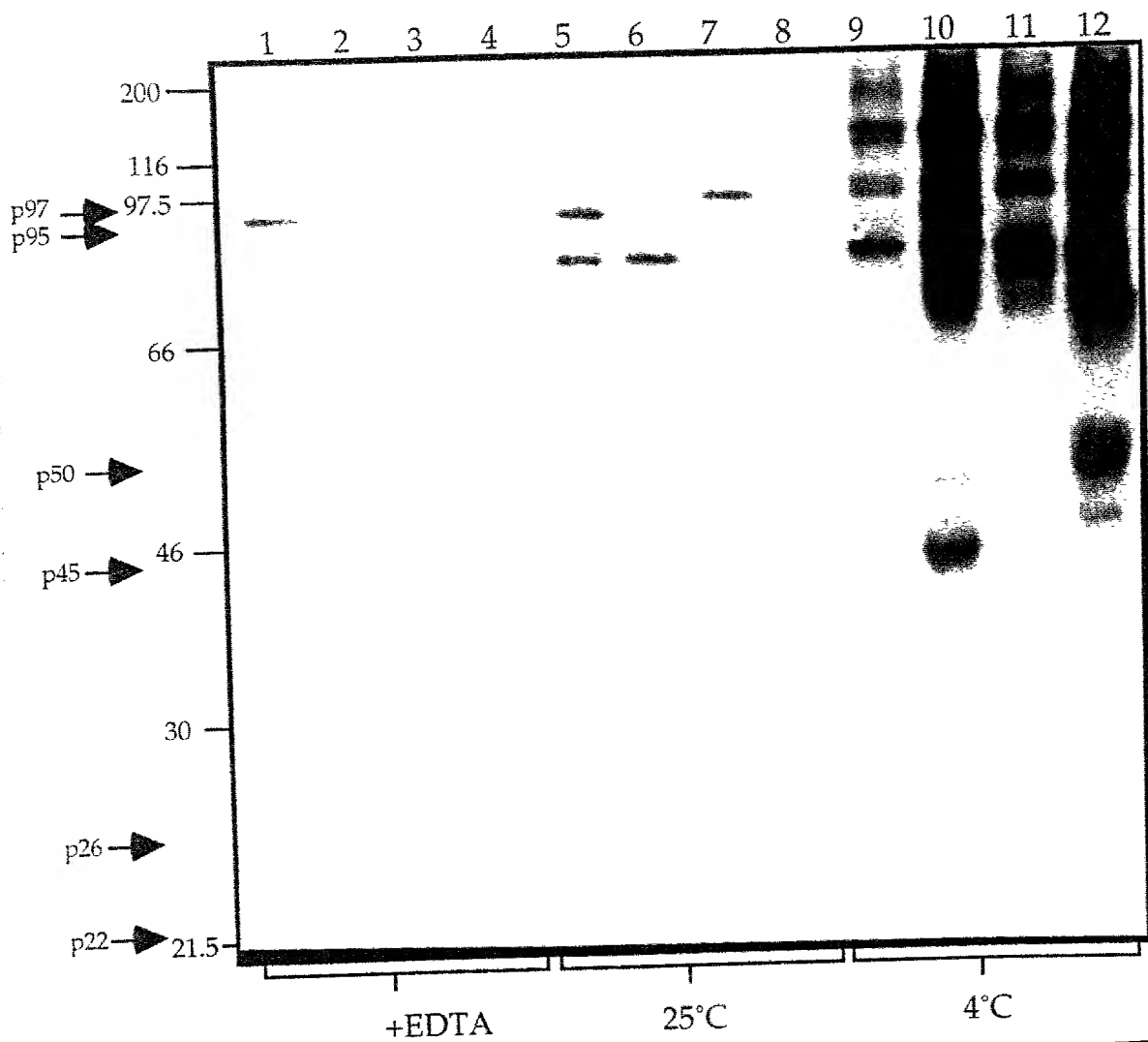


**Figure 20A**

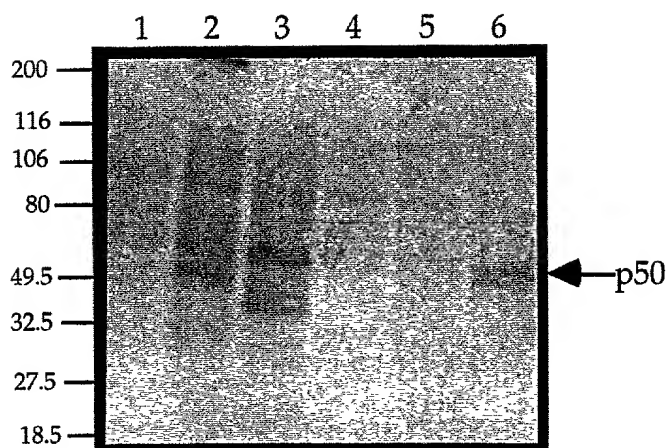
**Figure 20B**



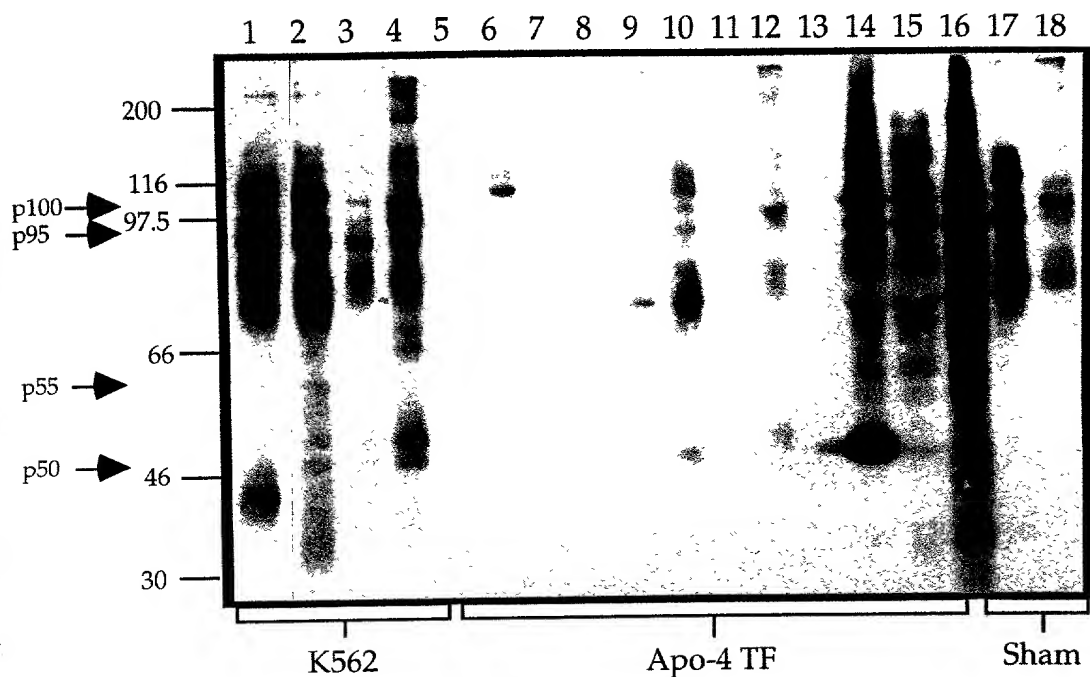
**Figure 21**



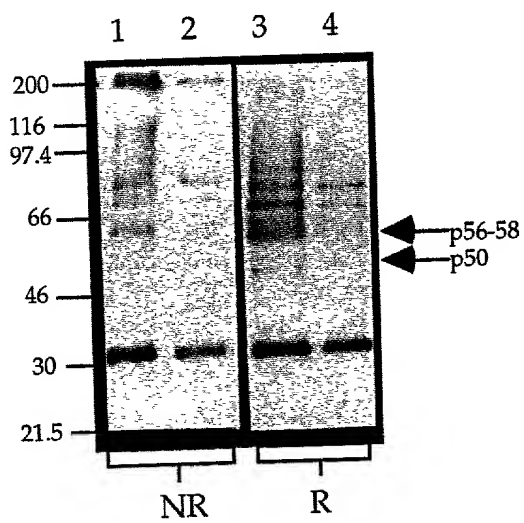
**Figure 22**



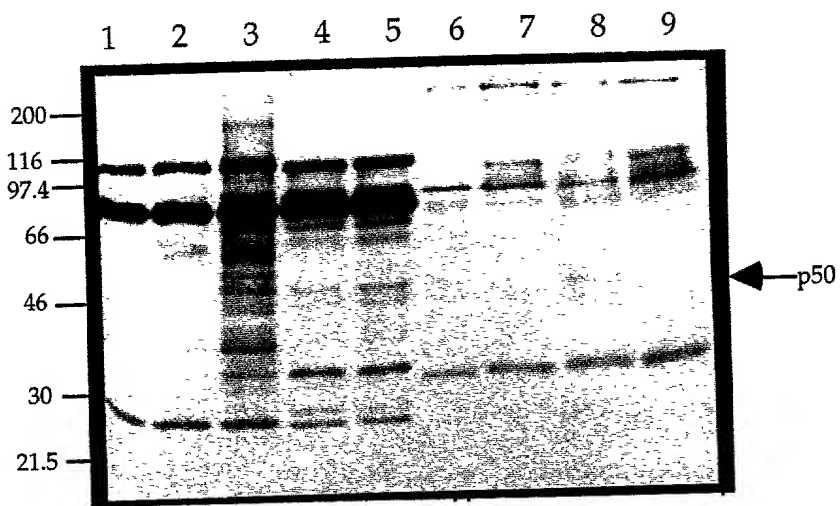
**Figure 23**



**Figure 24**



**Figure 25A**



**Figure 25B**

H2 starting at second methionine - 321 bp, predicted weight = 17.4Kd + 1 N-glycosylation site + 20.4 Kd.

Figure 26A

Splice sites for peptide  
MYPIMEYSCSDRNLVLIYGILLIYIYIGNLNMKKEQNKCFTTPDSRMVFIIIFIQQRGLDSKSLQEINL  
YFCEGFYTSMQLYKKVIRKLHKITQWTRTPQNQSEVEIA 107

Figure 26B

Start	Exon No.	Exon Position	Exon Length	Intron No.	Intron Position	Intron Length
@88 bp	78.3	@74-180	106 bp	79.1	@181-529	349 bp
	79.1	@530-654	125 bp	79.4	@655-720	66 bp
	79.4	@721-769	49 bp	79.55	@770-875	105 bp
	79.55	@876-893	18 bp	79.75	@894-932	39 bp
	79.85	@933-966	33 bp			

Hydrophobicity Scale KD; Candidate membrane-spanning segments:

Certain 1 12- 32 1.8833

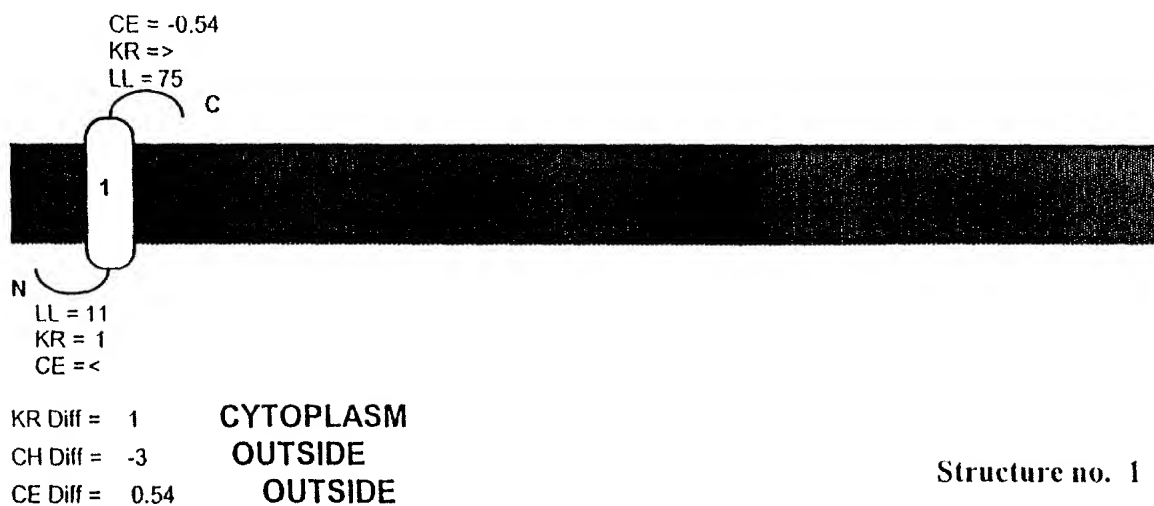
Figure 26C



# **Predicted TM structure**

> : Too long to be significant  
 < : Too short to be significant  
 LI : Loop length  
 KR : Number of Lys and Arg

KR Diff : Positive charge difference  
 CE : Net charge energy  
 CE Diff : Net charge difference  
 CH Diff : Charge difference over N-term segments



**Figure 26D**

1997-1998		1998-1999		1999-2000		2000-2001		2001-2002		2002-2003		2003-2004		2004-2005		2005-2006		2006-2007		2007-2008		2008-2009		2009-2010		2010-2011		2011-2012		2012-2013		2013-2014		2014-2015		2015-2016		2016-2017		2017-2018		2018-2019		2019-2020		2020-2021		2021-2022		2022-2023		2023-2024		2024-2025		2025-2026		2026-2027		2027-2028		2028-2029		2029-2030		2030-2031		2031-2032		2032-2033		2033-2034		2034-2035		2035-2036		2036-2037		2037-2038		2038-2039		2039-2040		2040-2041		2041-2042		2042-2043		2043-2044		2044-2045		2045-2046		2046-2047		2047-2048		2048-2049		2049-2050		2050-2051		2051-2052		2052-2053		2053-2054		2054-2055		2055-2056		2056-2057		2057-2058		2058-2059		2059-2060		2060-2061		2061-2062		2062-2063		2063-2064		2064-2065		2065-2066		2066-2067		2067-2068		2068-2069		2069-2070		2070-2071		2071-2072		2072-2073		2073-2074		2074-2075		2075-2076		2076-2077		2077-2078		2078-2079		2079-2080		2080-2081		2081-2082		2082-2083		2083-2084		2084-2085		2085-2086		2086-2087		2087-2088		2088-2089		2089-2090		2090-2091		2091-2092		2092-2093		2093-2094		2094-2095		2095-2096		2096-2097		2097-2098		2098-2099		2099-2100		2100-2101		2101-2102		2102-2103		2103-2104		2104-2105		2105-2106		2106-2107		2107-2108		2108-2109		2109-2110		2110-2111		2111-2112		2112-2113		2113-2114		2114-2115		2115-2116		2116-2117		2117-2118		2118-2119		2119-2120		2120-2121		2121-2122		2122-2123		2123-2124		2124-2125		2125-2126		2126-2127		2127-2128		2128-2129		2129-2130		2130-2131		2131-2132		2132-2133		2133-2134		2134-2135		2135-2136		2136-2137		2137-2138		2138-2139		2139-2140		2140-2141		2141-2142		2142-2143		2143-2144		2144-2145		2145-2146		2146-2147		2147-2148		2148-2149		2149-2150		2150-2151		2151-2152		2152-2153		2153-2154		2154-2155		2155-2156		2156-2157		2157-2158		2158-2159		2159-2160		2160-2161		2161-2162		2162-2163		2163-2164		2164-2165		2165-2166		2166-2167		2167-2168		2168-2169		2169-2170		2170-2171		2171-2172		2172-2173		2173-2174		2174-2175		2175-2176		2176-2177		2177-2178		2178-2179		2179-2180		2180-2181		2181-2182		2182-2183		2183-2184		2184-2185		2185-2186		2186-2187		2187-2188		2188-2189		2189-2190		2190-2191		2191-2192		2192-2193		2193-2194		2194-2195		2195-2196		2196-2197		2197-2198		2198-2199		2199-2200		2200-2201		2201-2202		2202-2203		2203-2204		2204-2205		2205-2206		2206-2207		2207-2208		2208-2209		2209-2210		2210-2211		2211-2212		2212-2213		2213-2214		2214-2215		2215-2216		2216-2217		2217-2218		2218-2219		2219-2220		2220-2221		2221-2222		2222-2223		2223-2224	
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```

P1                               Begin TM1 (R)
+30                               |                               P2
MYPIMEYSCSD RNLVLIYGIL LIYIYIGNLN VARHFSMKTP VARSNIKLIL 80
TNNVKWLHKK GFASSWKLVK NOTLLCTPSM QLLCCLHPEM GNDFPNGKET 130
                               P3
ERCYLSAPFV KSVFLSLCFP GHNVGSLFHM ADDLGRAMES LVSVMTDEEG 180
AEKMFYNSRF PHGFYNIHTT KRIRQKEFTR NKSIFLRRVV VLYCRFQKFL 230
SLLLFCKQWQ VLHVYAIVQK SYKKTTCKIL IAKKLAISLY GTHFGLFKNL 280
KQLKRKNYKG KRKKRNGQVV KLRTQVCTII RNTPKPKRGR NSMRSRVRCK 330
LI 332 (302aa in predicted polypeptide)

```

**Figure 1**

Certain	1	41-61	1.9073
Putative	2	101-121	0.8052
Certain	3	132-152	1.2552
Putative	4	217-237	1.1833
Putative	5	254-274	0.9240

CYT-EXT difference: 0.13: -> Orientation: **N-out**

Downloaded from <http://www.jstor.org/stable/2346229> on Tue, 20 Jun 2017 12:02:05 UTC

0996364-09301  
T03259-4929660

> : Too long to be significant  
< : Too short to be significant  
LI : Loop length  
KR : Number of Lys and Arg

KR Diff : Positive charge difference  
CE : Net charge energy  
CE Diff : Net charge difference  
CH Diff : Charge difference over N-term segments

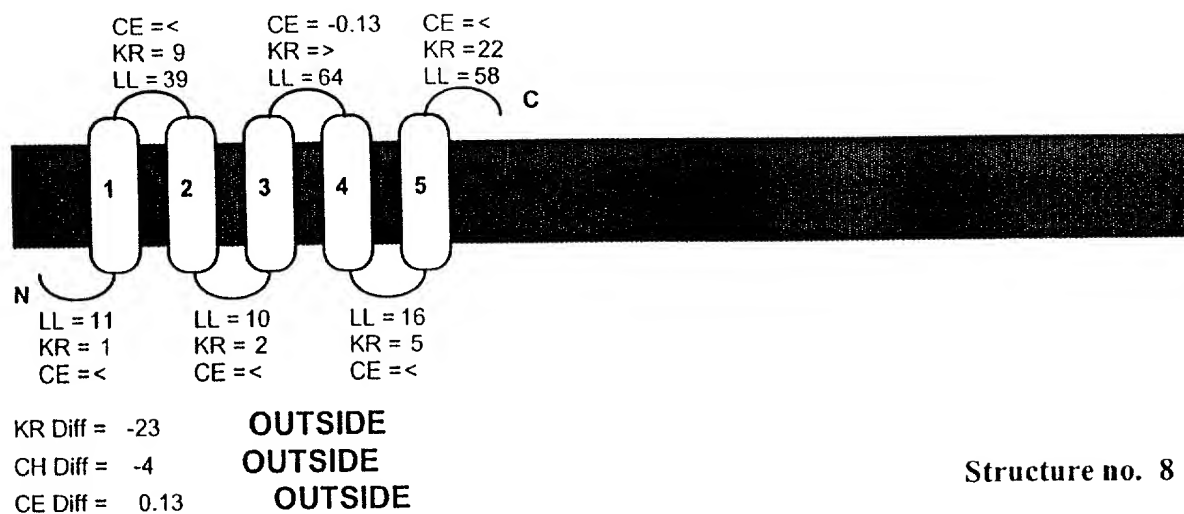


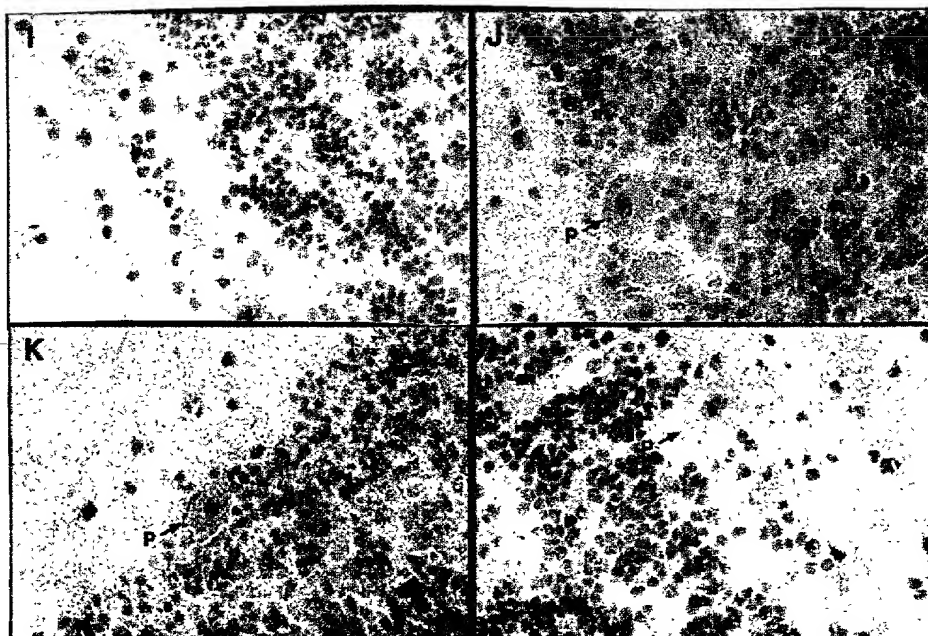
Figure 27C

108260-49299650



Figure 28

0966264.09201  
T0920"4929960



**Figure 28 (cont'd)**

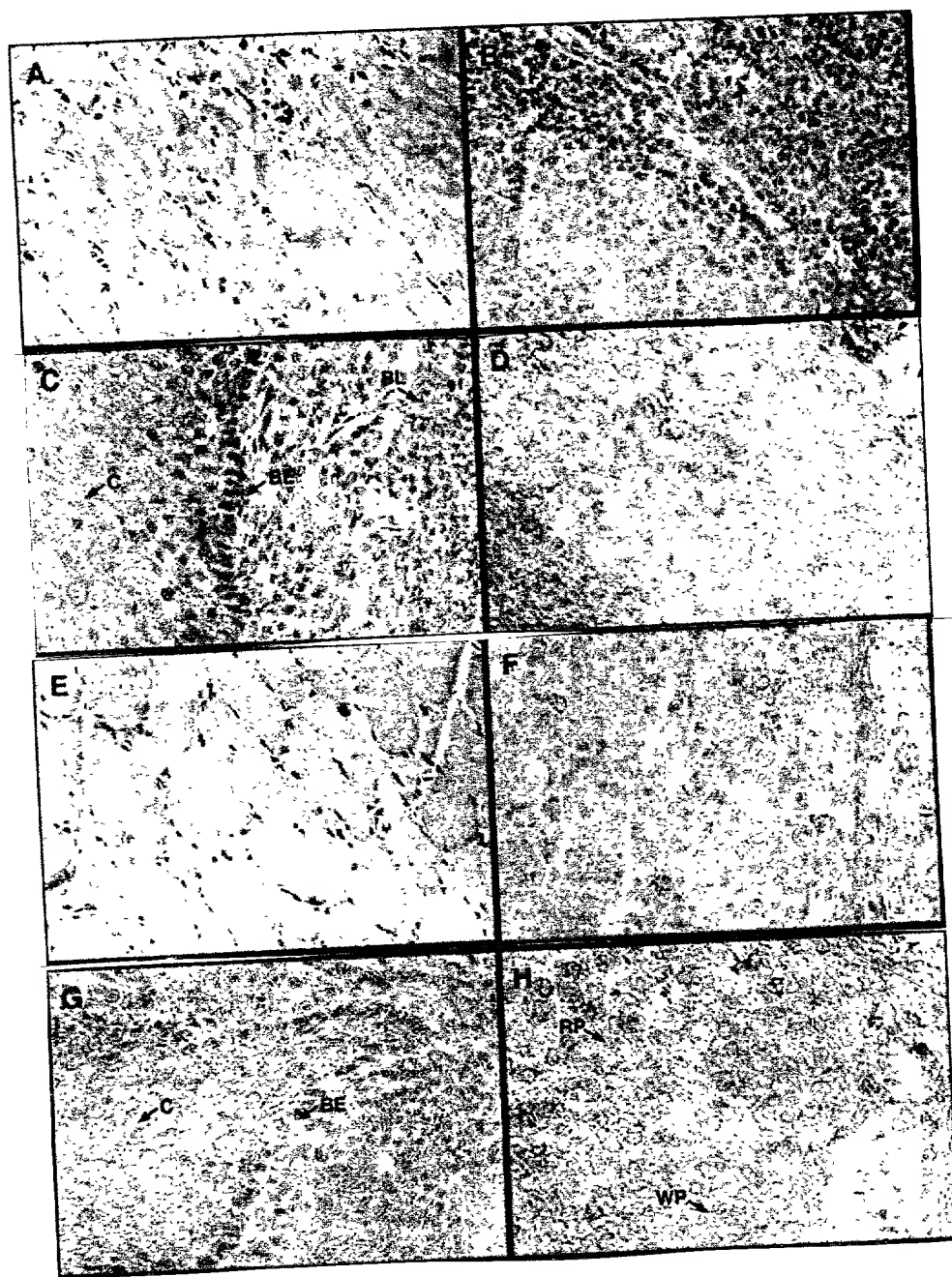
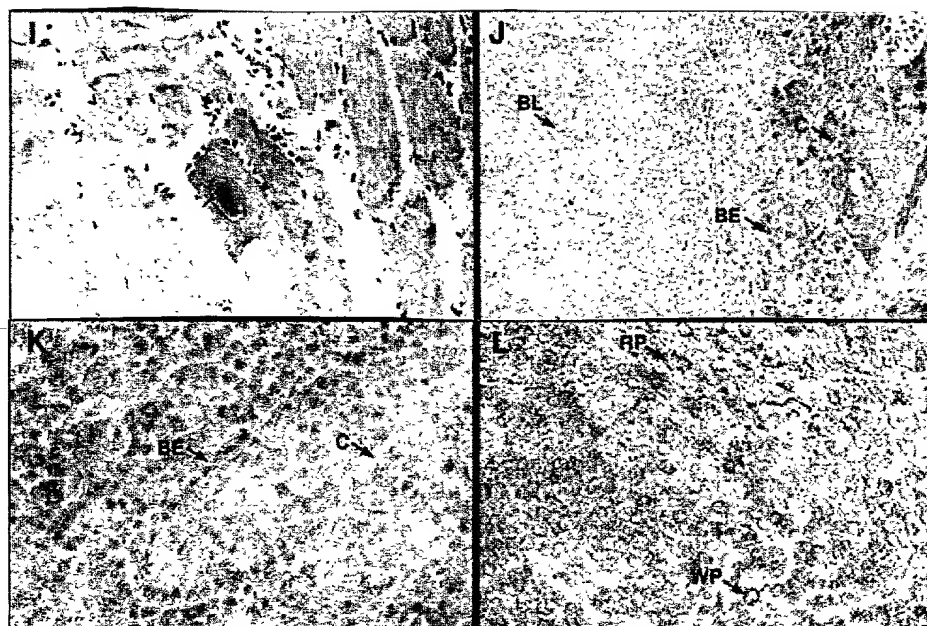


Figure 29



**Figure 29 (cont'd)**

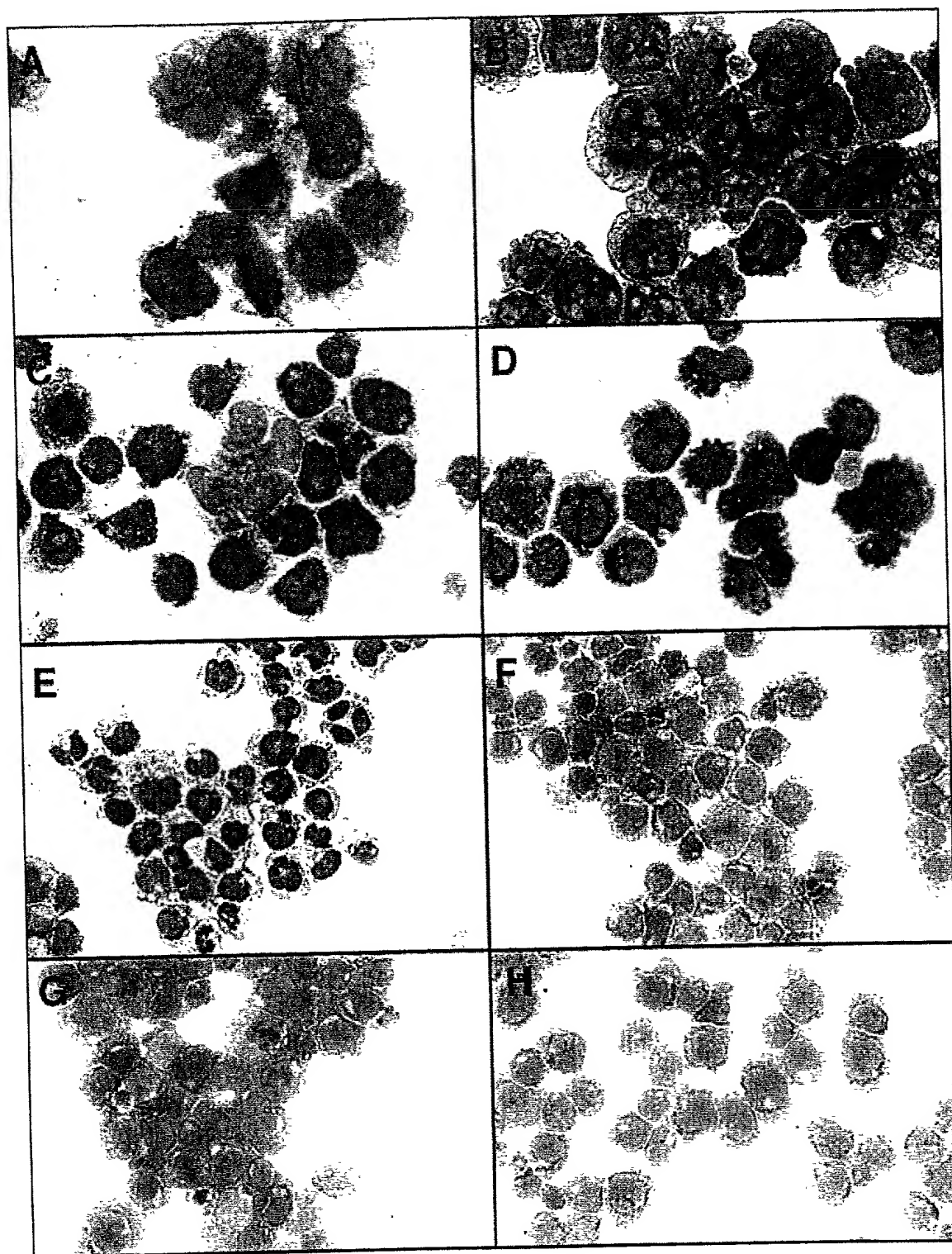


Figure 30



0966264-092801

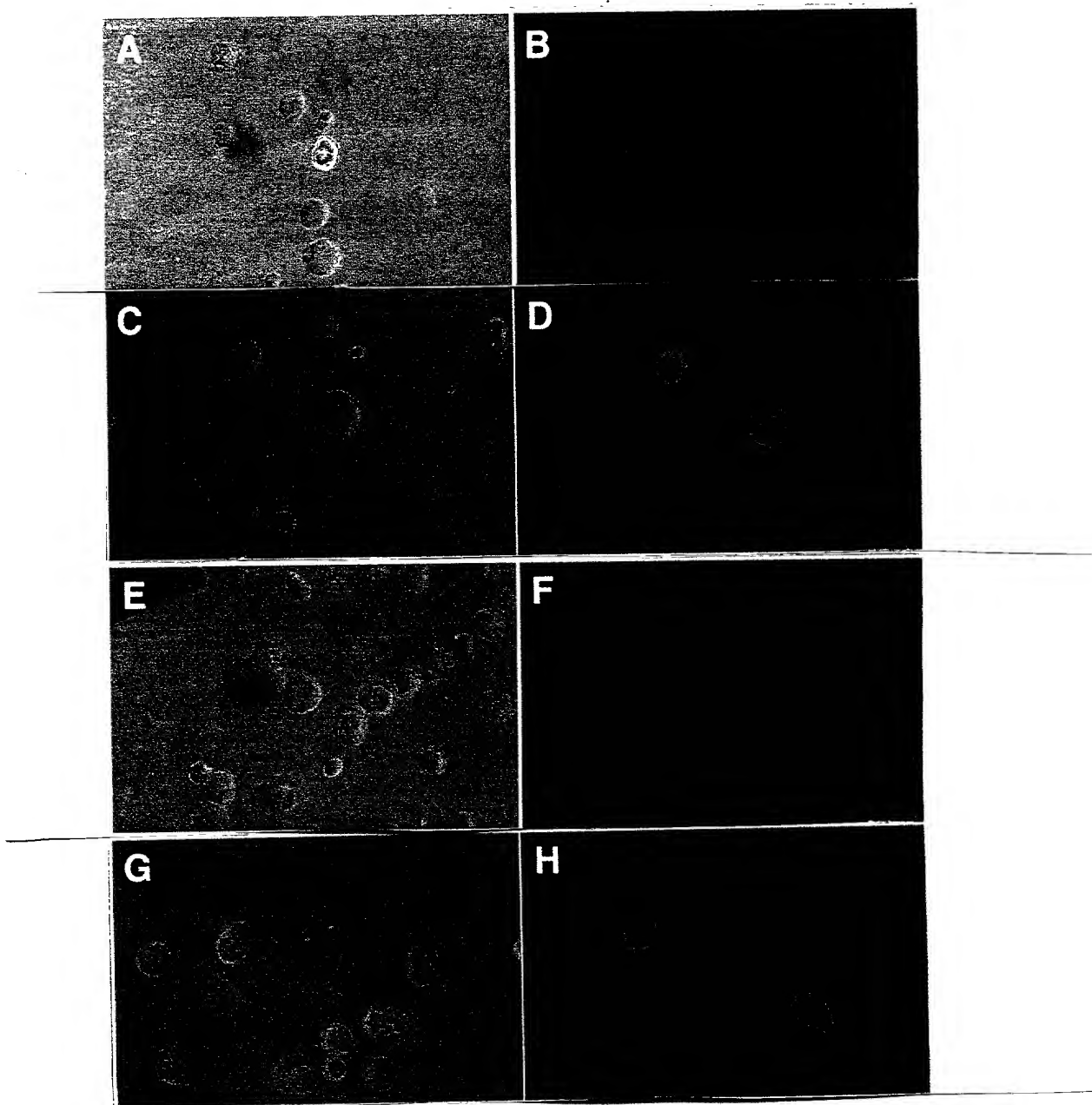
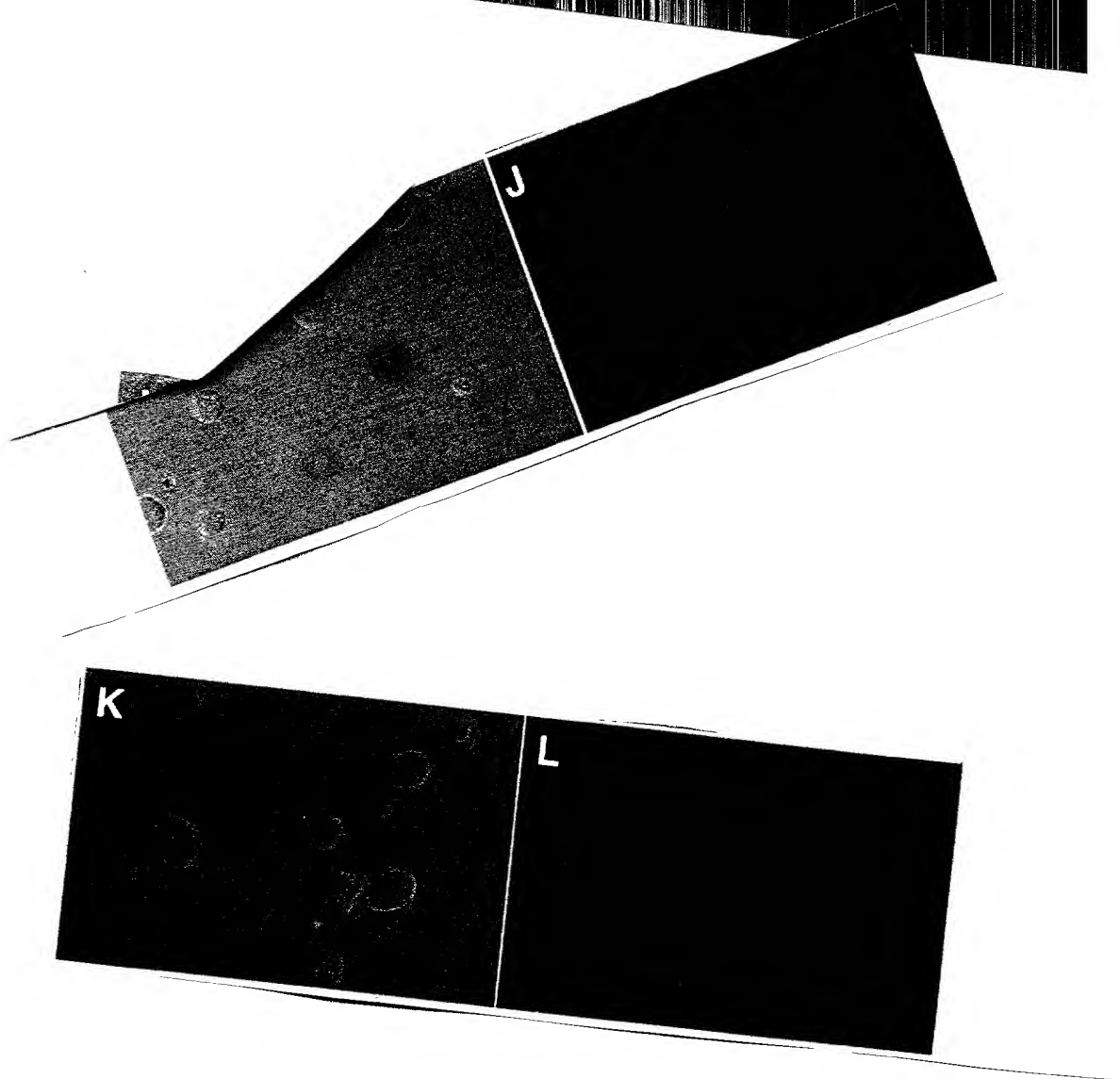


Figure 31

0956364.09301  
T09260"49293660



**Figure 31 (cont'd)**

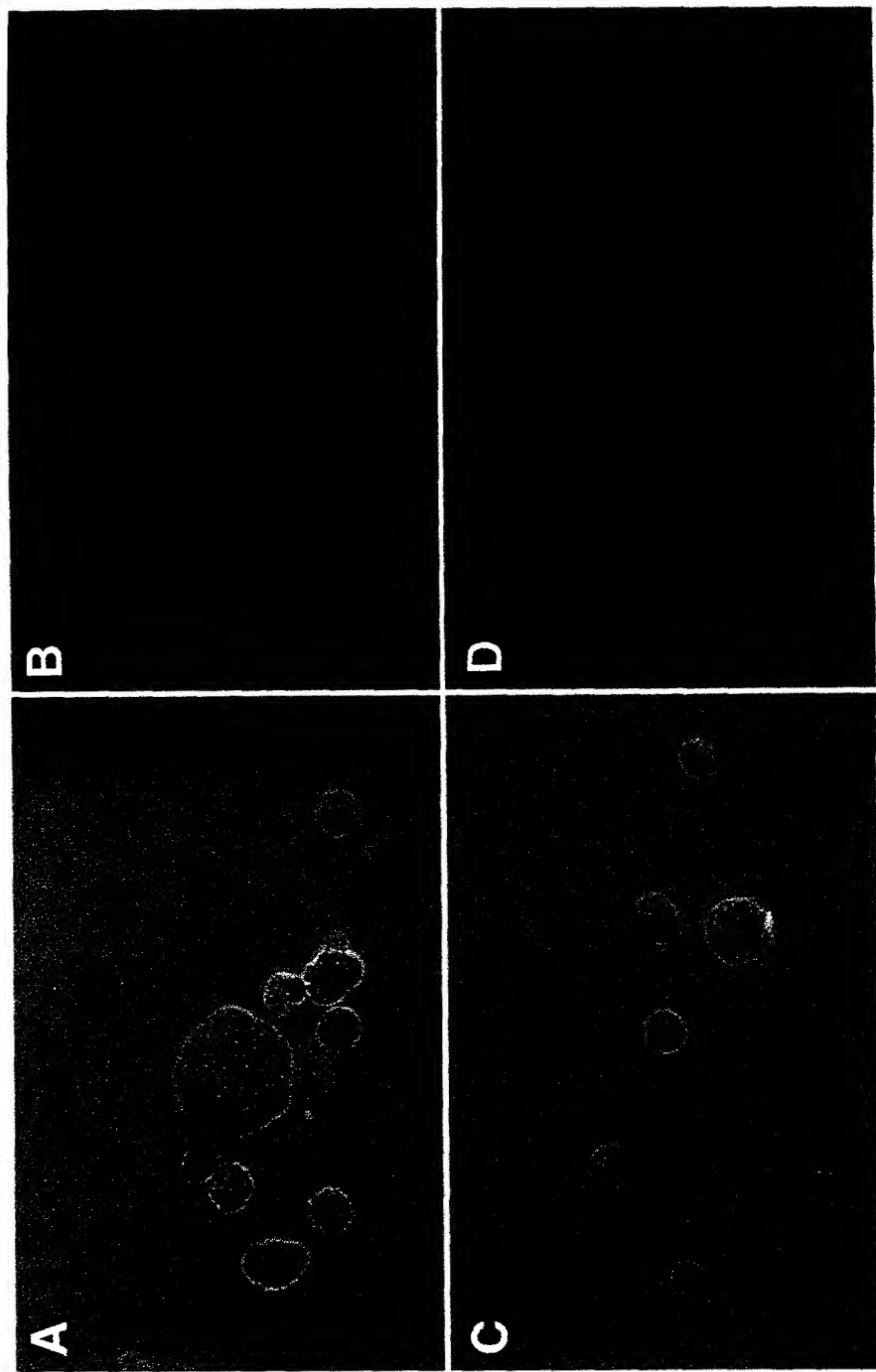


Figure 32

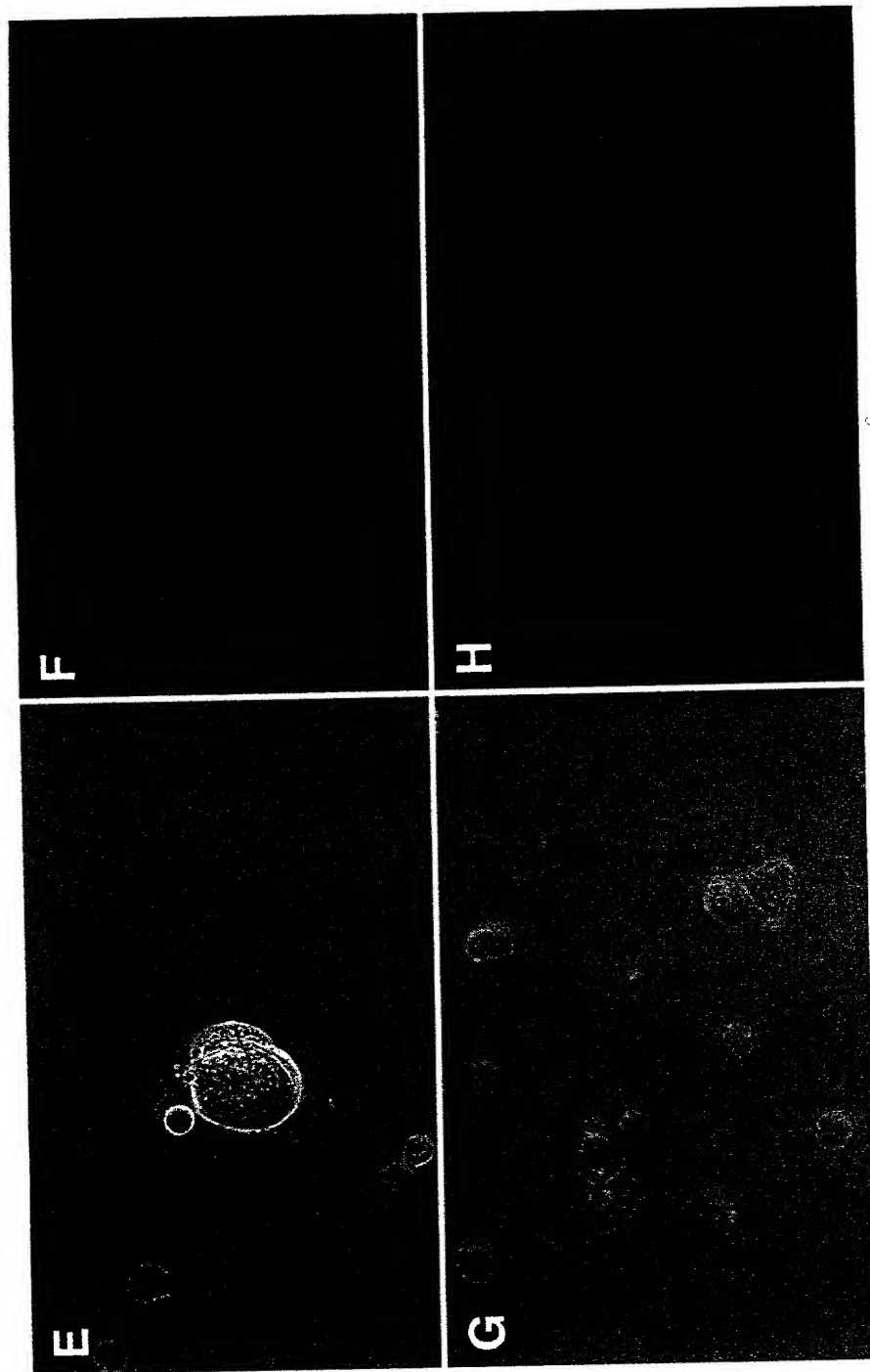


Figure 32 (cont'd)

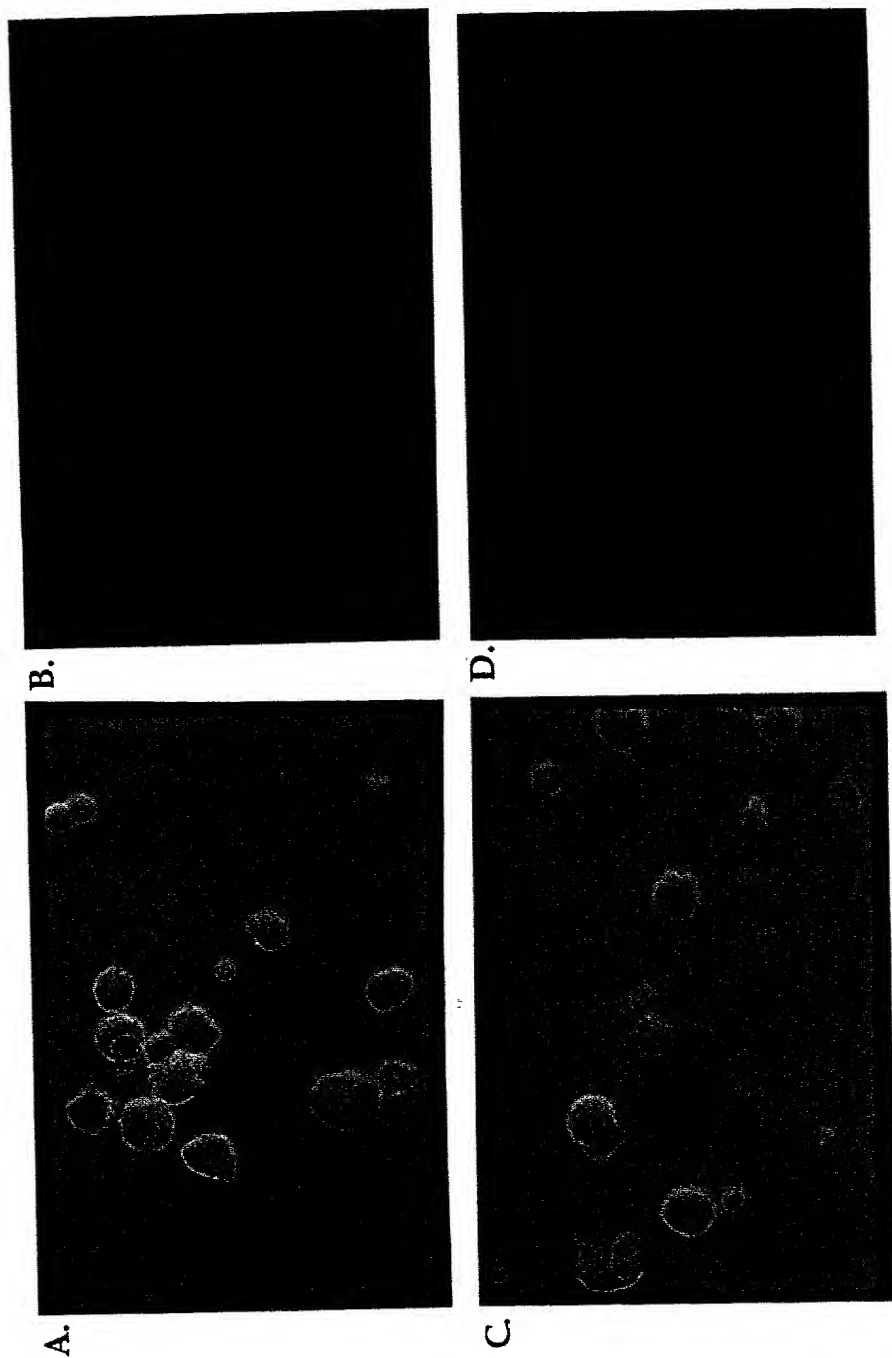


Figure 33

**Additional Oligonucleotide primers used for apo-dystrophin-4  
southern blotting and sequencing**

**FORWARD**

GTT CGT TAA TAC AAG TAG	F2.3(@28)	(SEQ ID NO 15)
GCC AAG GTG GAA AAG ATG	F2.2(@73)	(SEQ ID NO 16)
CCA GTA GCC TGA TCC AAC	F3.2(@208)	(SEQ ID NO 17)
GGC TTC ATT AAT AAG	F3.1(@257)	(SEQ ID NO 18)
GGC AAA GAA ACA GAG TG	F4.2(@379)	(SEQ ID NO 19)
CAG GAC ACA ATG TAG GA	F4.1(@449)	(SEQ ID NO 20)
GTT ATA AAG AAA GAA TTA TAA AG	FJn(@846)	(SEQ ID NO 21)
GAA AAT AAC GCA ATG GAC	F5.1(@875)	(SEQ ID NO 22)

**REVERSE**

GAT GGG ATA CAT CTT TTC C	R6.1(@99)	(SEQ ID NO 23)
CAA GCT ACA TTC AGG TTC CC	F2.2R(@188)	(SEQ ID NO 24)
GGA CTC CAT CGC TCT GCC	R4.1(@510)	(SEQ ID NO 25)
GAC TTA GAA ACT ACT G	R3.4(@694)	(SEQ ID NO 26)
ATA GAC GTG TAA AAC CTG C	R2.1(@735)	(SEQ ID NO 27)
AAC TGT TAT AAA TTT TTA	RSP2(@848)	(SEQ ID NO 28)
CTT TTT CCT TTA TAA TTC TTT C	R2.3o(@875)	(SEQ ID NO 29)

**Figure 34**

-----

## An Additional Splice Product Predicted From The Apo-4 Gene

A second potential theoretical splice product which retains exon 78.3 is shown below.

**H2 p1-124 spliced product =351 bp, 117 amino acids + 10 from vector + 1 N-glycosylation site; predicted weight = 21.9 Kd**

### Figure 35A

#### Peptide Generated

MFVNTTKVEKMYPIMEYSCSDRNLVLIYGILLIYIYIGNLNMKKEQNKCFITTPDSRMVFII  
FIQQRGLDSKSLQEINLYFCEGFYTSMLYKKVIRKLHKITQWTRTPQNPQSEVEIA (117  
amino acids) (SEQ ID NO 30)

### Figure 35B

Start	Exon No.	Exon Position	Exon Length	Intron No.	Intron Position	Intron Length
@26 bp	78.1	@16-41	26 bp	78.3	@42-74	33 bp
	78.3	@75-181	106 bp	79.1	@182-530	349 bp
	79.1	@531-655	125 bp	79.4	@656-721	66 bp
	79.4	@722-770	49 bp	79.55	@771-876	105 bp
	79.55	@877-894	18 bp	79.75	@895-933	39 bp
	79.85	@934- 967	33 bp			

Hydrophobicity Scale KD; Candidate membrane-spanning segments:

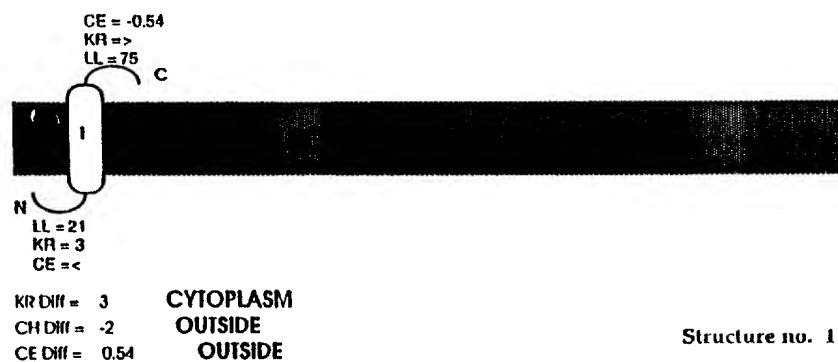
Certain 1 22- 42 1.8833

### Figure 35C

**Predicted TM structure**

>: Too long to be significative  
 <: Too short to be significative  
 U: Loop length  
 KR: Number of Lys and Arg

KR Diff: Positive charge difference  
 CE: Net charge energy  
 CE Diff: Net charge difference  
 CH Diff: Charge difference over N-term segments



**Figure 35D**



# **Nucleic Acid Subsequence Sites Identified In Apo-4**

<u><b>Motif</b></u>	<u><b>Position</b></u>	<u><b>Significance</b></u>
<b>CpG</b>	<b>-7, (+28, +106)</b>	<b>DNA methylation site</b>
<b>CAAT</b>	<b>-132, (+127, +131)</b>	<b>Binding of CAAT factors</b>
<b>TATAAT (5/6)</b>	<b>-120, -114, (+10)</b>	<b>TFIID Binding site</b>
<b>TATA</b>	<b>-154</b>	<b>Binds RNA polymerase II and TFIID</b>
<b>CCATTCA</b>	<b>-162, -131</b>	<b>Cap Site I</b>
<b>TATCAGT</b>	<b>+12, (+25)</b>	<b>Cap Site II</b>
<b>TGGCTGCAAGCCCAA (10/14)</b>	<b>-57, (+41)</b>	<b>Binds CTF/NF-I protein</b>
<b>GTGATGG</b>	<b>-140, -4, +11, +32</b>	<b>Eucaryotic Transcription Initiation Site</b>

**Figure 36**

General Information		Demographics		Clinical History		Physical Examination		Laboratory Studies		Imaging Studies		Treatment		Outcome			
Item	Value	Item	Value	Item	Value	Item	Value	Item	Value	Item	Value	Item	Value	Item	Value		
Age	65	Sex	Male	Chief Complaint	Shortness of breath	Weight	180 lbs	Height	70 in	BMI	30.0	ECG	Normal	ECG	Normal	ECG	Normal
Medical History	None	Current Medications	None	Duration of Symptoms	2 weeks	Smoking Status	Former	Alcohol Consumption	Occasional	Exercise Tolerance	Decreased	Chest X-ray	Normal	Chest X-ray	Normal	Chest X-ray	Normal
Family History	None	Previous Illnesses	None	Associated Symptoms	Chest pain	Diagnosis	Coronary Artery Disease	Prognosis	Good	Prognosis	Good	Prognosis	Good	Prognosis	Good	Prognosis	Good

```

Begin TM1 (R)
P1          |          P2
MFVNTSREKV INQSLIAKVE KMYPIMEYSCSD RNLVLLYGIL LIYIYIGNLN VARHFSMK60
TPVARSNIKL ILTNNVKWLH KKGFASSWKL VKNQTLLCTP SMQLLCCLHP EMGNDFPNGK 120
P3
ETERCYLSAP FVKSVFLSLC FPGHNVGSLF HMADDLGRAM ESLVSMTDE EGAEKMFYNS180
RFPHGFYNIH TTKRIRQKEF TRNKSIFLRR VVVLYCRFQ FLSLLLFCKQ WQVLHVYAIV 240
QKSYKKTTCK ILIAKLAIS LYGTHFGLFK NLKQLKRKNY KGKRKRNGQ VVKLRTQVCT 300
IIRNTPKPKR GRNSMRSRVR CKLI (324 amino acids) (SEQ ID NO 31)

```

**Figure 37A**

Certain	1	33- 53	1.9073
Putative	2	93- 113	0.8052
Certain	3	124- 144	1.2552
Putative	4	209- 229	1.1833
Putative	5	246- 266	0.9240

### Figure 37B

K+R difference: -19; -> Orientation: **N-out**; Charge-difference over N-terminal Membr. segs.

(±15 residues): -3; -> Orientation: **N-out**

CYT-EXT profile (neg. values indicate cytoplasmic preference): < < < < -0.13 <

CYT-EXT difference: 0.13

-> Orientation: **N-out**

II. Transmembrane segments included in structure 7: **1 3 4 5**; Loop lengths: 32 70 64 16  
58

K+R profile: 5 > 22 > 5; K+R difference: 22 -> Orientation: **N-in**

Charge-difference over N-terminal Membr. segs. (±15 residues): -3; -> Orientation: **N-out**

CYT-EXT profile (neg. values indicate cytoplasmic preference): < -0.13 < -0.26 <

CYT-EXT difference: 0.13; -> Orientation: **N-out**

### Figure 37B (cont'd)

— — — — —

09956264-092801  
199504-092801

## TopPred predicts a cytoplasmic N-terminus for four TM domains

> : Too long to be significative  
 < : Too short to be significative  
 U : Loop length  
 KR : Number of Lys and Arg

KR Diff : Positive charge difference  
 CE : Net charge energy  
 CE Diff : Net charge difference  
 CH Diff : Charge difference over N-term segments

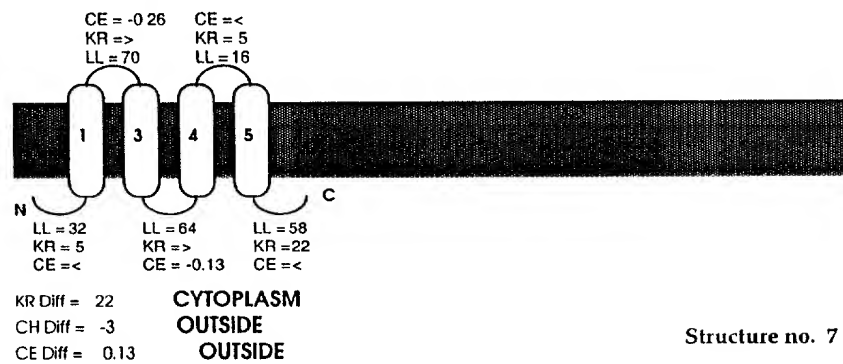
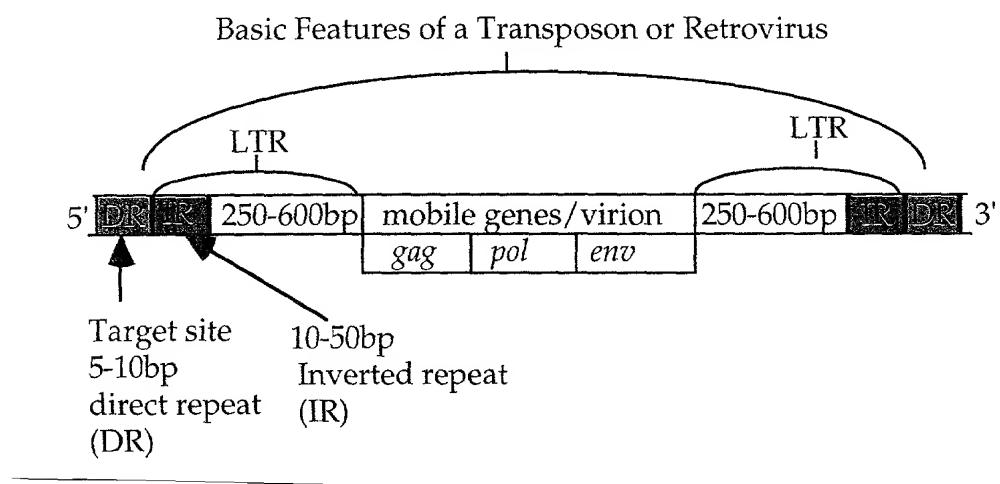
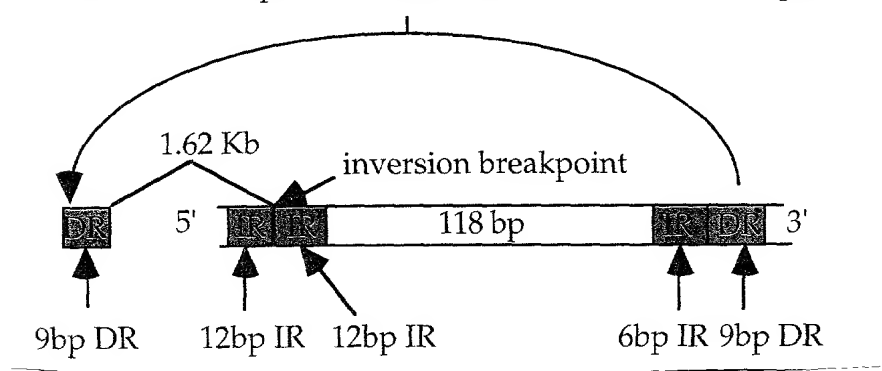


Figure 37C

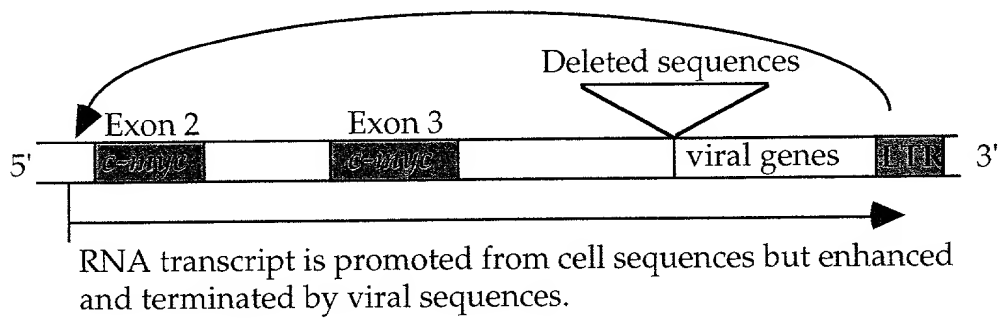


**Figure 38A**

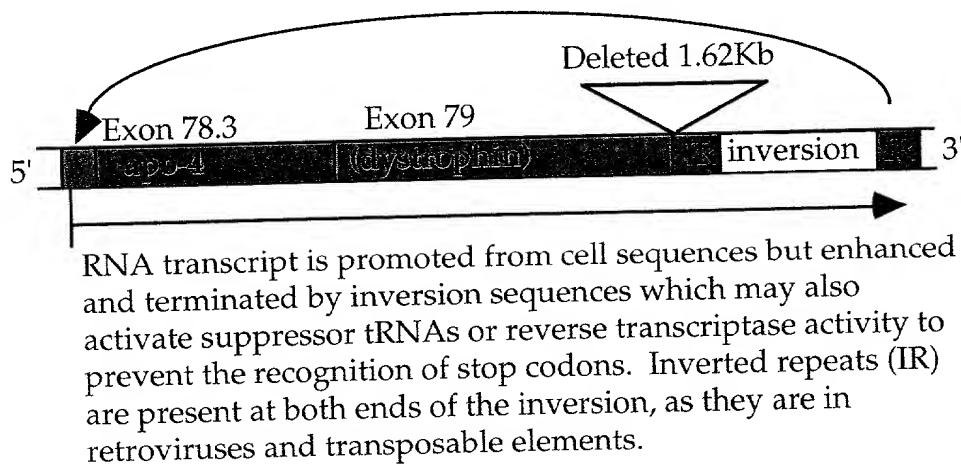
Structure of the apo-4 inversion element before rearrangement



**Figure 38B**



**Figure 39A**



**Figure 39B**